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ON THE EXCRETION OF KYNURENIC ACID.[1]

By LAFAYETTE B. MENDEL and HOLMES C. JACKSON, Ph.B.

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ALTHOUGH it is nearly half a century since Liebig discovered kynurenic acid in the urine of the dog, and this compound has long been assigned the constitution of an oxyquinoline-carboxylic acid,[2] there is much investigation yet demanded regarding its antecedents and origin in the metabolic processes of the body. The occurrence of kynurenic acid in the animal organism is interesting, because with the exception of α-methylquinoline recently isolated by Aldrich and Jones[3] from the anal secretion of Mephitis mephitica (common American skunk), it is, so far as we recall, the only quinoline compound discovered in connection with the animal body. Furthermore, the study of kynurenic acid production is important, because of the light which it promises to throw upon the transformations going on in the system, upon the constitution of the proteids from which the compound is derived, and possibly upon the physiological behavior of compounds like many of the alkaloids related to quinoline derivatives.

The early investigations on kynurenic acid can scarcely demand detailed consideration at present, since in the absence of satisfactory

[1] A preliminary account of some of the experiments described in this paper was presented at a meeting of the American Physiological Society, December 28, 1897.

[2] Schmiedeberg and Schultzen: Ann. Chem. Pharm., 1872, clxiv, p. 155. Kretschy: Berichte d. deutsch. chem. Gesell., 1879, xii, p. 1673; Monatshefte für Chemie, 1881, ii, p. 57.

[3] Aldrich and Jones: Journal of experimental medicine, 1897, ii, p. 439.

analytical methods the separation of uric acid (and possibly other substances) from the acid investigated was not accomplished.[1] It cannot be assumed that kynurenic acid completely replaces uric acid in the urine of the dog, inasmuch as the experiments of Solomin[2] have shown that both acids may occur together under appropriate conditions, and our own experience leads to a similar conclusion. Solomin found that although the uric acid nitrogen (determined by the Ludwig-Salkowski method) forms only a very small fraction of the total nitrogen excreted, the quantity of uric acid estimated per kilo of body weight may be as large as 0.01 gram, which corresponds with the average uric acid output per kilo in man. In the case of dogs in nitrogenous equilibrium, numerous experiments in this laboratory have given a considerably smaller excretion (0.003–0.004 gram per kilo).[3] The higher figures obtained by Solomin are perhaps attributable to the rather large quantities of proteid fed.[4]

Regarding the immediate origin of kynurenic acid, little of a positive character is to be found in physiological literature. Its close relation to the diet, and its ready production after the ingestion of meat, have frequently been pointed out. Thus Schmidt[5] believed to have found kynurenic acid excretion to be greatest after feeding meat, and least with a bread diet, a milk diet yielding intermediate results. His figures for the various dietaries are, however, by no means comparable, since the quantities of the typical foodstuffs ingested in the three periods were not at all equivalent.[6] The experiments of Schmidt and of Rosenhain,[7] planned to observe the

[1] Cf. for example, Voit and Riederer: Zeitschrift für Biologie, 1865, i, p. 315.

[2] Solomin: Zeitschr. f. physiol. Chemie, 1897, xiii, p. 497.

[3] Cf. Chittenden: Journal of physiology, 1891, xii, p. 220. Chittenden and Gies: This journal, 1898, i, p. 1.

[4] The daily diet of the 9-kilo dog consisted of meat, 400 grams; milk, 250 c.c.; and NaCl, 10 grams (*loc. cit.* p. 498). Regarding the increase in uric acid excretion following the ingestion of proteid, cf. Schultze: Arch. f. d. ges. Physiol., 1889, xlv, p. 401: Herter and Smith: New York medical journal, June 4, 1892: Hopkins: Schaefer's Physiology, 1898, i, p. 594.

[5] Schmidt: Ueber das Verhalten einiger Chinolinderivate im Thierkörper mit Rücksicht auf die Bildung von Kynurensäure. Inaugural-Dissertation. Königsberg, 1884. (Jaffé's laboratory.)

[6] The dog was fed, (*a*) meat, 1 kilo, (*b*) milk, 2 litres, (*c*) bread, 1 pound, respectively, per day.

[7] Rosenhain: Beiträge zur Kenntniss der Kynurensäurebildung im Thierkörper. Inaugural-Dissertation, Königsberg, 1886. (Jaffé's laboratory.) Cf. also, R. Cohn: Zeitschr. f. physiol. Chemie, 1895, xx, p. 210.

possible production of kynurenic acid from various quinoline derivatives [1] introduced directly into the organism, gave only negative results. The observations of Rosenhain and of Haagen,[2] in which a decrease of from thirty to fifty per cent of kynurenic acid was obtained after administration of intestinal antiseptics (*e. g.* salol, thymol, naphthalin) suggested a connection between intestinal putrefaction and kynurenic acid excretion. There is, however, no satisfactory evidence in these experiments that kynurenic acid has its origin in the decomposition going on in the intestine, since no data are given regarding the direct action of the drugs administered upon the food utilization and body metabolism. Thus it seems quite possible in view of our experiments that the diminished kynurenic acid excretion observed after naphthalin administration, for example, is to be attributed to poorer absorption of the proteid fed. It may also be recalled in this connection how many drugs, *e. g.*, antipyrin, antifebrin, salicylates, alcohol, exert a direct influence upon the production of uric acid; and accordingly similar specific effects may have been at work in Haagen's experiments. Again, Baumann [3] observed an undiminished excretion of kynurenic acid in a dog in which several days' starving and repeated doses of calomel had freed the intestine from putrefactive processes as shown by the absence of ethereal sulphates in the urine; while Haagen failed to find any decrease in kynurenic acid excretion after administering large doses of iodoform, which exerts a pronounced action upon the putrefactive processes in the intestine.[4] In this connection we may point out that Nuttall and Thierfelder [5] have lately demonstrated the possible origin of aromatic oxyacids in tissue metabolism, since they have found them in the urine of animals which were entirely free from all bacteria. It seems desirable to emphasize the preceding facts because the experiments of Haagen have repeatedly been misinterpreted and quoted in evidence of the intestinal origin of kynurenic acid,[6] although Haagen

[1] *E. g.* carbostyril, quinaldin, oxymethyl-quinoline, kynurin, antipyrin.

[2] HAAGEN: Ueber den Einfluss der Darmfaulniss auf die Enstehung der Kynurensäure beim Hunde. Inaugural-Dissertation, Königsberg, 1887. (Jaffé's laboratory.)

[3] BAUMANN: Zeitschr. f. physiol. Chemie, 1886, x, p. 131.

[4] Cf. MORAX: Zeitschr. f. physiol. Chemie, 1886, x, p. 321.

[5] NUTTALL and THIERFELDER: Zeitschr. f. physiol. Chemie, 1895, xxi, p. 109; 1896, xxii, p. 62.

[6] Cf. for example, HAUSER: Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 3; also, NEUMEISTER: Lehrbuch der physiol. Chemie, 2te Auflage,

has carefully avoided such an interpretation of his observations.[1] Finally, the experiments of Capaldi[2] have given additional evidence against the assumed intestinal origin of kynurenic acid.

In considering the immediate antecedents of kynurenic acid tyrosin is at once suggested. The behavior of this aromatic compound with reference to its possible synthesis to oxyquinoline-carboxylic acid in the body has been investigated by Hauser[3] and Solomin,[4] both of whom failed to obtain evidence of any direct relationship between the two substances.

Plan of present investigation. — The present investigation is an attempt to ascertain something more definite regarding the conditions which determine and modify kynurenic acid production and excretion. Unless otherwise stated, the data have been obtained with dogs. The animals were kept in suitable roomy cages which permit the separate collection of urine and fæces, and stand in a light, well-ventilated space. It was not found necessary to resort to catheterization, since the periods of observation always extended over more than one day and the animals soon became accustomed to discharge their urine with considerable regularity. The nitrogen was determined in the urine and diet by the Kjeldahl method; sugar, when present, was estimated by titration with Fehling's or Purdy's solution,[5] and kynurenic acid was found by the method of Capaldi,[6] which has proved very satisfactory. The product thus obtained always responded to Jaffé's test[7] and was crystalline; in a few urines a very small quantity of an amorphous substance was precipitated,

1897, p. 721. "Eine ältere, von Baumann stammende Angabe, dass die Quantität der Kynurensäure von den Fäulnissprocessen im Darm unabhängig sei, scheint durch die neueren Untersuchungen widerlegt zu sein."

[1] Cf. HAAGEN: *loc. cit.*, p. 26. ". . . so ist es zweifelhaft, ob die nach anderen Antisepticis, besonders nach Naphthalin gefundene Verminderung der Kynurensäure auf Beschränkung der Darmfäulniss, oder ob sie nicht vielmehr auf anderen Umständen beruht."

[2] CAPALDI: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 87.

[3] HAUSER: Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 1.

[4] SOLOMIN: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 497.

[5] Cf. J. BISHOP TINGLE: American chemical journal, 1898, xx, p. 126.

[6] CAPALDI: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 92. Solomin (*ibid.* p. 498 note) recovered by this method 99 per cent of 0.210 gram kynurenic acid added to urine. The following figures show average duplicates obtained by us from a dog's urine containing small quantities: (*a*) 0.0852 gram. (*b*) 0.0872 gram.

[7] JAFFÉ: Zeitschr. f. physiol. Chemie, 1883, vii, p. 399.

which failed to give the characteristic reaction. As an immediate test for kynurenic acid — in the urine — the bromine water reaction, first recommended by Baumann,[1] was frequently found useful. Bromine, as is well known, usually gives an insoluble yellow precipitate when added to dog's urine, the composition of the precipitate depending upon the presence of phenol bodies, indol, or kynurenic acid. With a little experience it becomes easy to make use of the reaction in judging the relative amounts of kynurenic acid, since the latter ordinarily composes (as tetrabromkynurin[2]) by far the greater part of the precipitate formed.

Experiments on dogs. — For these experiments commercial cracker-dust containing as an average 1.46 per cent nitrogen was obtained in large quantity and kept in glass-stoppered bottles. This constituted the *carbohydrate* food fed. The *fat* used was a good quality of lard practically free from nitrogen. The other foodstuffs used will be referred to in the protocols.

The following experiments demonstrate the formation of kynurenic acid after the ingestion of various proteids of both vegetable and animal origin. The "dog biscuit" used was a commercial preparation containing dried meat, carbohydrates (sugar-beet), etc.; the albumin was commercial albumen e sanguine; the vegetable proteid was crystallized edestin (phytovitellin) prepared from hemp seed after the manner already described by one of us;[3] the Witte's "pepton" was the widely used product made up almost entirely of proteoses (from fibrin). The latter preparation contained 14 per cent N. A mixture of inorganic salts as recommended by J. Munk[4] was daily added to the diet in experiment C.

Various investigators have demonstrated that the proteoses and peptones may show caloric and nutritive values equivalent to those of the proteids from which they originate.[5] Several of our experiments (B, C, D) show a characteristic excretion of kynurenic acid after repeated feeding of proteoses (Witte's "pepton"). No disturbances of the gastro-intestinal tract (as with "Somatose," p. 9) were observed with this product.

[1] BAUMANN: Zeitschr. f. physiol. Chemie, 1877, i, p. 62.
[2] BRIEGER: Zeitschr. f. physiol. Chemie, 1881, iv, p. 89.
[3] CHITTENDEN and MENDEL: Journal of physiology, 1894, xvii, p. 49.
[4] J. MUNK: Virchow's Arch. f. d. exper. Pathologie, cxxxii, p. 102.
[5] Cf. MUNK AND EWALD: Die Ernährung, 1895, p. 34.

DOG A.

Date.	Body Weight.	Urine.				Food.
1897.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Kynurenic Acid. grams	grams
Nov. 22	9.0	110	1026	Acid	trace	carbohyd., 50; fat, 50; water, 200.
23	8.9	165	1006	"	none	" " "
24	8.8	160	1011	"	"	" " "
25	8.8	195	1013	"	"	" " "
26	8.7	175	1016	"	"	" " "
27	8.7	190	1027	Alkaline	0.012	casein,[1] 50; carbohyd., 50; fat, 40; water, 200.
28	8.8	230	1012	Acid	none	casein,[2] " " " "
29	8.7	125	1013	"	"	casein,[2] " " " "
30	8.9	195	1024	"	0.035	casein, " " " "
Dec. 1	8.9	165	1014	"	none	casein,[2] 100 " " "
2	8.9	250	1015	"	0.014	casein,[2] " " " "
3	8.8	70	1037	"	0.036	meat, 300; (little fat).
4	8.9	190	1046	"	0.124	" 400; " "
5	8.9	275	1045	"	0.103	" " " "

Dec.	6	8.9	205	1048	Acid	0.178	**meat**, 400; (little fat).
	7	9.0	110	1047	"	0.058	carbohyd., 50; fat, 50; water, (200).
	8	8.9	60	1041	"	none	" " "
	9	8.9	90	1017	"	"	" " "
	10	8.9	160	1017	"	"	**casein**, 150; carbohyd., 25; fat, 12; water, 75.
	11	8.9	320	1021	"	0.023	" 240; " 50; " 25; " 100.
	12	9.0	400	1017	"	0.057	" 300; " 50; " 50; " 200.
	13	9.0	160	1018	"	0.032	" 75; " 25; " 25; " 100.
	14	9.1	420	1018	"	0.121	" 350; " 50; " 50; " 200.
	15	9.1	65	1030	"	0.017	carbohyd., 50; fat, 50; water, (100).
	16	9.1	85	1020	"	0.005	" " "
	17	9.1	120	1018	"	none	none (water).
	18	8.9	85	1033	"	"	" "
	19	8.8	70	1025	"	"	" "
	20	8.6	65	1025	"	"	" "
	21	8.7	50	1027	"	"	" "

[1] The casein in this and following experiments was either a commercial preparation, or casein prepared from milk, reprecipitated once and fed moist. The moist casein contained 25 to 30 per cent dry substance.

[2] Moist casein.

DOG B.

Date.	Body Weight.	Urine.				Food.
1897.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Kynurenic Acid grams	grams
Dec 2	10.0	330	1012	Acid	none	none (water)
3	10.0	none	....	....	....	" "
4	9.9	185	1024	"	0.152	**meat**, 300; little fat.
5	9.8	400	1044	"	0.119	**meat**, 400; little fat.
6	9.5	43	1048	"	0.025	**liver**, 150.
7	9.6	240	1048	"	0.345	none (water).
8	...	none	....	....	0.093 [1]	" "
9	9.4	175	1039	Acid	0.093	" "
10	9.0	none	....	....	0.097 [1]	" "
11	...	"	....	....	0.097	" "
12	8.5	250	1034	"	0.097	" "
13	8.6	85	1034	"	0.081	carbohyd., 50; fat, 50; water, 200.
14	8.7	60	1048	"	none	" 100; " 100; " 300.
15	8.7	225	1016	"	"	" 75, " 75; " 150.

Dec. 16	8.6	115	1024	Alkaline	none	{ carbohyd., 25; fat, 25; water. { "**Somatose**," 25.[2]
17	8.6	225	?	Acid	"	{ carbohyd., 30; fat, 25; water. { "**Somatose**," 50.
18	8.4	230	?	Alkaline	"	{ carbohyd., 30; fat, 25; water. { "**Somatose**," 50.
19	8.4	65	1024	Acid	"	"**Somatose**," 14, etc.
20	8.5	95	1024	"	"	carbohyd., 25; water, 50.
21	8.5	100	1028	"	"	" 125; " 200.

[1] The figures given for the daily kynurenic acid output of this period are calculated from the total amount excreted, there being no urine voided on part of the days.

[2] "Somatose" is a commercial product prepared by digestion of meat and composed largely of proteoses (70 to 80 per cent) with small quantities of peptone (R. H. CHITTENDEN: Dietetic and hygienic gazette, x, p. 47). The sample used contained 13.9 per cent N. In this animal it produced vomiting and diarrhœa, and doubtless only a portion of the material fed was absorbed. Irritation of the gastro-intestinal tract by large quantities of similar digestion products has frequently been observed. Cf. MUNK u. EWALD: Die Ernahrung des gesunden und kranken Menschen, 1895, p. 427; also TREUPEL: Münchner med. Wochenschr., 1898, p. 611.

DOG C.

Date.	Body Weight.	Urine.					Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	grams
Mar. 21	9.9	179	1063	Acid	....	0.010	**dog biscuit.**
22	9.9	222	1058	"	....	0.036	"
23	9.9	none	....	"	....	....	"
24	10.1	380	1038	"	12.08	0.011	"
25	10.0	460	1029	Alkaline	8.59	0.099	**albumin**, 50; carbohyd., 75; fat, 40.
26	10.0	455	1037	Acid	11.68	0.168	" 75; " "
27	10.0	325	1040	"	8.53	0.187	" 100; " "
28	10.0	400	1035	"	10.27	0.106	" 100; " "
29	9.9	174	1048	"	5.60	0.053	" 50; " "
30	10.0	430	1029	"	13.42	0.125	" 50; " "
31	10.0	554	1029	"	17.94	0.141	**edestin**, 75; " "
April 1	9.9	385	1029	"	13.08	0.137	" 100; " "
2	10.1	415	1022	"	9.32	0.191	" 100; " "
3	10.1	350	1018	"	6.69	0.009	**albumin**, 15; " "
4	10.0	222	1050	"	9.61	0.018	" 15; " "
5	10.0	276	1030	Alkaline	8.17	0.428	**Witte's "pepton,"** 75; carbohyd., 100; fat, 40.

DOG D.

Date.	Body Weight.	Urine.				Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Kynurenic Acid. grams	grams
April 17	8.3	202	1027	Acid	0.028	albumin, 15; carbohyd., 50; fat, 40; water.
18	8.3	105	1020	"	trace	" " " "
19	8.3	180	1023	"	none	" " " "
20	8.3	150	1030	"	0.017	" " " "
21	8.3	188	1041	"	0.333	albumin, 15; carbohyd., 50; fat, 40; Witte's "pepton," 75; water; daily.
22	8.4	312	1050	"	0.276	
23	8.3	143	1055	Alkaline	0.182	
24	8.6	550	1015	Neutral	trace	albumin, 15; carbohyd., 50; fat, 40; water.
25	8.5	264	1014	Alkaline	none	" " " "
26	8.4	258	1019	Acid	"	" " " "

DOG E

Date.	Body Weight.	Urine.					Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	grams
May 20	9.9	366	1014	Acid	5.02	0.044	**albumin**, 15; carbohyd., 50; fat, 40; water.[1]
21	9.9	195	1014	Neutral	2.05	0.039	" " " "
22	9.9	510	1011	Alkaline	3.57	0.041	" " " "
23	9.8	500	1009	Neutral	2.77	none	" " " "
24	9.8	820	1016	Alkaline	5.02	0.175	" 80; " " "
25	9.8	665	1016	"	8.05	0.149	" " " " "
26	9.8	650	1017	"	6.83	0.200	" " " " "
27	9.8	340	1012	"	2.98	0.055	" 15; " " "
28	9.8	455	1010	"	2.32	0.028	" " " " "
29	9.7	410	1023	"	7.48	0.064	" 40; **gelatin**, 40; carbohyd., 50; fat, 40; water.
30	9.5	440	1017	"	1.47	none	" " " " "
31	9.5	560	1023	"	12.17	0.108	" " " " "
June 1	9.4	335	1011	"	2.73	0.080	" 15; " " " "
2	9.5	410	1015	"	4.00	0.027	" 15; " " " "
3	9.4	480	1017	"	7.77	none	**gelatin**, 80; carbohyd., 50; fat, 40; water.
4	9.3	435	1019	"	7.74	"	" " " "
5	9.3	240	1024	"	5.99	"	" " " "
6	9.4	560	1009	"	3.19	"	**albumin**, 15; " " "
7	9.5	350	1010	"	1.84	"	" 15; " " "
8	9.5	425	1014	"	2.70	0.049	" 40; " " "
9	9.5	750	1010	"	3.39	0.167	" 80; " " "

[1] Ad libitum.

The results obtained in the preceding experiment are summarized in the following table, which gives the daily averages for various feeding periods.

DOG E. — SUMMARY.

(Giving daily averages for various feeding periods.)

Date.	Urine.		Food.	
1898.	Nitrogen.	Kynurenic Acid.	Nitrogen.	Proteid.
	grams			
May 21–23	2.79	0.027	1.75	albumin, 15.
24–26	6.63	0.175	9.36	" 80.
27, 28	2.65	0.044	1.75	" 15.
29–31	7.04	0.057	10.20	albumin, 40; gelatin, 40.
June 1, 2	3.36	0.053	1.75	" 15.
3–5	7.17	none	11.04	gelatin, 80.
6, 7	2.51	none	1.75	albumin, 15.

In view of the peculiar chemical and physiological behavior of gelatin in contrast to the ordinary proteids, some experiments were undertaken with this albuminoid (Dogs F, G, H). Commercial gelatin, containing 13.8 per cent N, was fed, it being eagerly eaten when mixed with water and the other food stuffs as indicated. Occasionally 1 to 2 grams of Liebig's extract of beef were added to improve the flavor, while a mixture of inorganic salts as recommended by J. Munk [1] was daily given with the food. The following dog F, which had sometime previously been used in a phlorhizin experiment, had been fed very large quantities of casein on the days immediately preceding the experiment.

At the conclusion of the experiment on the dog H, the animal was starved for eighteen days. Body-weight fell from 7.6 kilos to 6.0 kilos. During this period 1220 c.c. of urine, containing 107 mgr. kynurenic acid, were eliminated.

[1] J. Munk: Virchow's Arch. f. d. exper. Pathologie, cxxxii, p. 102.

DOG F.

Date.	Body Weight.	Urine.					Food.[1]
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	grams
Feb. 1	7.2	138	1043	Acid	6.11	0.073	**casein**, 25; fat, 50; carbohyd., 50.
2	7.2	77	1032	"	2.52	0.028	" " "
3	7.2	248	1015	"	3.51	none	" " "
4	7.1	112	1030	"	3.79	"	**gelatin**, 35.
5	7.2	230	1027	"	7.15	"	" 40.
6	7.2	156	1028	"	5.65	"	" 45.
7	7.0	112	1022	Alkaline	....	0.008	**albumin**, 20; carbohyd., 35.
8	7.0	155	1026	"	....	0.046	" 30; "
9	7.0	228	1025	"	3.72	0.060	" 40; "
10	7.0	477	1018	"	7.23	0.104	" 50; "
11	7.1	260	1033	"	3.26	0.138	" 60; " 10.
12	7.0	248	1050	"	10.16	0.076	" 60; **gelatin**, 35; carbohyd., 10.
13	6.9	198	1049	"	8.02	0.003	" " "
14	6.9	174	1044	"	9.57	0.021	" 50; " 20; " 35.
15	6.9	480	1028	Alkaline	10.15	0.123	" 60; " "
16	6.9	338	1020	"	9.71	0.072	" 40; fat, 40; " 50.
17	6.9	294	1017	"	.	none	" 30; " 30; "
18	6.9	258	1026	"	....	0.079	" " " "
19	7.0	455	1014	Neutral	....	none	" 25; " 40; "
20	7.0	275	1016	"	....	"	" " " "
21	7.0	298	1013	Alkaline	....	"	" " " "

[1] Water was given ad libitum.

DOG G.

Date.	Body Weight.	Urine.					Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	grams
Mar. 14	8.6	137	1046	Acid	....	0.085	none (water).
15	8.3	none	...	"	....	[1] 0.069	" "
16	8.3	150	1043	"	....	[1] 0.069	" "
17	8.0	70	1048	"	....	0.069	
18	8.0	211	1035	"	....	0.137	**albumin**. 20; carbohyd., 50; fat, 40; water.
19	8.0	170	1023	Neutral	....	0.023	" " " "
20	8.0	320	1012	Acid	....	0.028	" " " "
21	8.0	383	1015	"	....	0.050	" " " "
22	8.0	214	1021	"	....	0.068	" " " "
23	8.0	412	1017	"	....	0.075	" " 75; " "
24	8.0	345	1012	"	2.47	0.016	" 15; " " "
25	8.0	200	1018	"	2.07	0.025	" " " " "
26	8.0	146	1020	"	1.79	0.028	" " " " "
27	8.0	293	1021	"	3.55	0.024	" " " " "
28	8.0	275	1017	"	3.13	0.011	" " " " "
29	8.0	184	1054	"	8.16	none	" 15; **gelatin**, 75; carbohyd., 75; fat, 40; water.
30	8.1	258	1041	"	10.69	"	" " 100; " " "
31	8.2	624	1030	"	19.66	"	" " " " "
Apr. 1	8.0	548	1026	"	12.15	0.015	" 50; " 50; " " "
2	8.1	290	1038	"	5.96	0.053	**albumin**. 100; carbohyd., 75; fat, 40; water.
3	8.0	265	1043	"	7.61	0.091	" " "
4	8.1	288	1018	"	3.52	none	carbohyd., 75; fat, 40; water.
5	8.1	164	....	"	1.67	"	" "

[1] The figures given for the daily Kynurenic acid output of this period are calculated from the total amount excreted, there being no urine voided on one of the days.

DOG H.

Date.	Body Weight.	Urine.					Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	grams
Mar. 13	8.5	41	1033	Acid	1.18	none	none; water.
14	8.3	37	1031	"	1.08	"	" "
15	8.1	57		"	1.81	0.012	" "
16	8.0	41	1033	"	0.94	none	" "
17	7.9	none	....	....	1.35	0.015	" "
18	7.8	none	....	....	[1] 1.35	[1] 0.015	" "
19	7.8	60	1050	"	1.35	0.015	" "
20	7.6	160	1013	"	1.87	0.010	" "
21	7.5	128	1019	"	2.13	0.004	casein,[3] 100; fat, 100; water, 75.
22	7.6	105	1017	"	1.53	0.017	" 50; " "
23	7.6	94	1017	"	1.56	0.016	" " "
24	7.6	105	1023	"	2.32	0.026	" " "
25	7.7	136	1020	"	2.46	0.018	" " "
26	7.8	56	1031	"	1.47	0.011	" 75; " "

Mar. 27	7.8	116	1023	Acid	2.39	0.041	**casein,**[3] 75; fat, 100; water, 75.
28	7.8	300	?	"	....	none	" 75; **gelatin**, 35; fat, 100; water.
29	7.6	325	?	"	8.01	"	" " " "
30	7.6	290	?	Alkaline	....[2]	"	" 35; " 70; " "
31	7.6	270	1030	Acid	6.85	"	" " 70; " "
April 1	7.7	162	1031	"	5.53	0.061	" 350.
2	7.6	230	1035	"	8.68	0.137	" 375.
3	7.6	330	1024	"	8.81	0.028	" 350.
4	7.7	174	1044	"	9.11	0.017	" 175; **gelatin**, 30.
5	7.6	150	1047	"	8.27	none	" 90; " 45.
6	7.6	75	1040	"	3.97	"	fat, 100; " 15.
7	7.6	204	1022	"	....	0.013	**casein,**[3] 215; carbohyd., 50; fat, 25; **gelatin**, 25.
8	7.7	295	1023	"	....	0.034	" 300; **gelatin**, 30.

[1] Cf. note 1, Dog G.
[2] A small quantity of fæces became mixed with the urine and made an accurate urine-nitrogen determination impossible.
[3] Moist casein.

Discussion of the preceding analytical data. — So far as has been observed, all aromatic compounds found in the organism of the higher animals are derived either from benzene derivatives introduced into the system, or from the proteids, which must accordingly contain aromatic radicals. A synthesis of the latter in the animal body from carbohydrates or fats scarcely seems probable in view of the accumulated experimental data bearing on the problem.[1] The present study of kynurenic acid excretion lends additional force to this view as applied to quinoline derivatives. Thus kynurenic acid is almost always found in the urine during starvation, a condition in which body proteids form the source of the nitrogenous compounds excreted. This observation, repeatedly made (*c. g.* Dogs G, H, J, K), leaves little doubt that kynurenic acid is a true product of proteid katabolism, and for the most part, at least, is not dependent for its origin on the putrefactive processes in the intestine, — a possibility which has already been mentioned.

Quantitatively considered, kynurenic acid production bears a more or less direct relation to the variations in the decomposition of proteid material, whether the latter be the "tissue proteid" of a starving animal, or introduced as food and the kynurenic acid formed incidental to its metamorphosis. Moreover, our experiments furnish repeated evidence that kynurenic acid is a concomitant or direct product of accelerated proteid metabolism. There is no lack of evidence that the proteid molecule may break down in the body into a nitrogenous and non-nitrogenous portion. The former part is doubtless rapidly further broken down, oxidized and synthesized perhaps into various nitrogenous constituents of the urine. The non-nitrogenous moiety is less speedily eliminated. It may be converted into glycogen, or dextrose, or fat; and forming, as it does, the major part of the original molecule, it may be distributed to the tissues more slowly in proportion as they demand it.[2] The experiments of Feder[3] and of Reilly, Nolan and Lusk[4] indicate that most of the nitrogen of ingested meat is, on the other hand, eliminated within a few hours. It may be imagined, then, that kynurenic acid is one of the nitrogenous products derived from this rapidly eliminated nitrogenous radical of the original proteid; and

[1] Cf. Baumann: Zeitschr. f. physiol. Chemie, 1886, x, p. 123.
[2] Cf. Reilly, Nolan and Lusk: This journal, 1898, i, p. 404.
[3] Feder: Zeitschrift für Biologie, 1881, xvii, p. 541.
[4] Reilly, Nolan and Lusk: *loc. cit.*, p. 404 flg.

it scarcely seems unreasonable to assume that this acid represents the metabolic end-product of quinoline-yielding radicals in the molecule.[1] In this connection it will be remembered that R. Cohn[2] was able to obtain a pyridine derivative as a decomposition product of casein, thus demonstrating the possible presence of the pyridine ring in the constitution of the proteid molecule.

Granting conditions like those referred to, several probabilities are indicated. For example, it should follow that the extent of proteid katabolism going on should influence very largely the production of kynurenic acid. Our experiments with non-nitrogenous diet (fat and carbohydrate) are quite in harmony with this. Excessive proteid decomposition is avoided under such conditions, and kynurenic acid rapidly disappears from the urine. (Cf. Dog A, B.) Equally suggestive are the results obtained with a mixed diet containing only small amounts of proteid. The proteid-sparing effect of the other foodstuffs is here well brought out. In one animal (Dog A) an absence of kynurenic acid excretion during the first seven days of starvation was noted. The observation was an exceptional one, but perhaps not without significance, since the animal had an unusually well nourished appearance and the "fat" condition was remarkably persistent even during the later days of starvation. We are inclined to attribute the results to the rather exceptional condition of the animal, since it is well known that the extent of nitrogenous katabolism in inanition is modified in a pronounced way by the relative as well as absolute amount of body fat in the individual.[3]

Kynurenic acid excretion has also been investigated in three dogs which were kept in nitrogenous equilibrium on a fixed diet of meat, fat, and carbohydrates. The animals were used in a research carried out in this laboratory on the influence of borax and boric acid on nutrition. Through the kindness of Dr. Gies we have obtained equivalent portions of each day's urine and have examined them for kynurenic acid, the fractions of each period being united and analyzed collectively. Further data are taken from the tables already published.[4]

[1] Cf. also KRETSCHY: Monatshefte für Chemie, 1881, ii, p. 85.
[2] R. COHN: Zeitschr. f. physiol. Chemie, 1896, xxii, p. 171.
[3] Cf. MUNK and EWALD: Die Ernährung, 1895, pp. 22–23.
[4] CHITTENDEN and GIES: This journal, 1898, i, p. 1.

FIRST EXPERIMENT.

Period.	Nitrogen balance.	Uric acid.	Kynurenic acid.
(9 days).	grams		
Fore	−0.981	0.428	none
Borax (5 grams)	−1.928	0.411	0.101
After	−0.117	0.389	none

SECOND EXPERIMENT.

Period.	Nitrogen balance.	Uric acid.	Kynurenic acid.
(10 days).	grams		
Fore	+0.118	0.504	none
Boric acid (1–2 grams) . .	−1.506	0.386	trace
After	−0.980	0.508	none

THIRD EXPERIMENT.

Period.	Nitrogen balance.	Uric acid.	Kynurenic acid.
(8 days).	grams		
Normal	+0.429	0.315	none
Borax (2–5 grams) . . .	−0.801	0.293	none
After	+0.661	0.248	none
Boric acid (1–3 grams) . .	+2.174	0.310	none
After	+2.122	0.354	none
Borax (8 grams)	**−4.878**	**0.295**	**0.414**
After	−0.661	0.328	none

It will be observed from the tables that the only periods during which kynurenic acid appeared in the urine were those in which administration of borax or boric acid gave rise to a direct stimulation of proteid metabolism. This is particularly brought out in the third

experiment, during which a daily dose of eight grams of borax produced a nitrogen deficit of over four grams. Uric acid, however, was continually present in the urine of the dogs employed; and the results obtained are in harmony with the opinion that kynurenic acid excretion is a phenomenon accompanying pronounced stimulation of proteid katabolism rather than ordinary conditions of body equilibrium.

The gelatin experiments. — Eckhard [1] stated that after feeding gelatin to a dog he failed to find kynurenic acid in the urine. Rosenhain [2] likewise obtained no kynurenic acid after feeding two and a half pounds of gelatin and six pounds of bread to a large dog in the course of a week. After a meal of one kilo of meat, however, the same animal excreted as much as 0.995 gram of kynurenic acid during the succeeding twenty-four hours. No data are presented to show that the gelatin was absorbed satisfactorily.

The results obtained with gelatin feeding in the present investigation afford an interesting confirmation of previous observations. The experiments of J. Munk [3] have shown that in dogs over two-thirds of the required proteid of the diet may be replaced by gelatin with maintenance of nitrogenous equilibrium. Gelatin is as a rule readily digested and burned in the body, and has a pronounced proteid-sparing action like a typical non-proteid food. Lusk and his co-workers have recently shown that gelatin — like the proteids proper — may yield sixty per cent of sugar in the metabolic changes it undergoes in the body, as was evidenced by the amount of sugar excreted when the albuminoid was fed to a fasting animal in phlorhizin diabetes. Gelatin differs chemically from the ordinary proteids in that tyrosin has not been found among its decomposition products.[4] The absence of tyrosin is suggestive of a deficiency in aro-

[1] Eckhard: Ann. Chem. Pharm., 1856, xcvii, p. 358.

[2] Rosenhain: Beiträge zur Kenntniss der Kynurensäurebildung im Thierkörper. Inaugural-Dissertation. Königsberg, 1886, p. 8.

[3] J. Munk: Arch. f. d. ges. Physiol., 1894, lviii, p. 309.

[4] Halliburton: Schaefer's Physiology, 1898, i, p. 71. It may be added that K. B. Lehmann failed to accomplish proteid synthesis in rats by feeding them with gelatin and tyrosin (Sitzungsber. d. morphol.-physiol. Gesellsch. in München, 10 März, 1885). Contrary to the current statements, cf. Neumeister: Lehrbuch der physiol. Chemie, 1897, p. 63, pure gelatin gives a reaction with Millon's reagent which may perhaps be due to a far smaller proportion of aromatic radicals than is present in ordinary proteids. See Van Name: Journal of experimental medicine, 1897, ii, p. 128.

DOG J.

Date.	Body Weight.	Urine.						Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic acid. grams	Dextrose. grams	grams
Jan. 11	8.8	197	1029	Acid	3.90	0.023	....	none
12	8.5	....	....	....	....	0.008[1]	....	"
13	8.3	96	1033	"	2.54	0.008[1]	....	"
14	8.1	120	1034	"	3.64	0.078	....	"
15	8.0	....	....	....	....	0.070[1]	....	" water.
16	7.9	142	1030	"	4.91	0.070[1]	....	" "
17	7.7	73	1044	"	2.64	0.060	....	" "
18	7.5	172	1056	"	4.57	0.130	10.49	**phlorhizin**, 1; (three times a day).
19	7.5	300	1058	"	8.18	0.168	26.67	" "
20	7.3	360	1056	"	7.88	0.141	25.03	" "
21	7.2	226	1063	"	6.27	0.150	19.06	**casein**, 100; fat, 100; water, 75.
22	7.0	176	1037	"	3.82	0.063	....	" 50; " "
23	6.9	98	1025	"	2.04	0.029	....	" "
24	6.9	112	1017	"	1.78	0.059	....	" " fat, 100; water, 75.

Jan. 25	6.9	134	1016	Acid	1.56	0.030	....	**casein**, 50; fat, 100; water, 75.
26	6.9	76	1018	"	1.22	0.006	....	" 75; " 150; " 100.
27	7.0	127	1021	"	2.11	none	....	" 25; " 50; " 100.
28	6.9	265	1023	"	3.48	0.082	....	" 320; water.
29	7.0	370	1016	"	6.95	0.091	....	" 545; "
30	7.1	295	1021	"	7.83	0.122	....	" 574; "
31	7.2	335	1026	"	12.24	0.139	....	" 515; "

[1] Daily average calculated from two days' urine.

matic groups in the molecule, and it is not unlikely that this fact may account for the absence of the related compound kynurenic acid (oxyquinoline-carboxylic acid) when gelatin exclusively is fed. (Cf. Dogs E, F.) Out of 905 grams gelatin fed to small dogs in fourteen days we have obtained only

DOG J.—SUMMARY.

Period.	Urine.	
	Nitrogen	Kynurenic Acid
	grams	
Fasting, 3 days	7.5	0.200
Phlorhizin, 3 days	20.63	0.440
Casein, etc., 3 days	12.13	0.243

0.015 gram kynurenic acid, and that on a single day. Moreover, the nitrogen determinations in the urine (cf. Dogs E, F, G, H) give evidence of a ready absorption of the gelatin ingested; while the experiments including simultaneous feeding of typical proteids like

casein and albumin with the gelatin show that the latter exercises no *specific* action in preventing kynurenic acid excretion when sufficient proteid is given along with it. Indeed, the behavior of gelatin precisely resembles that of the carbohydrates and fats. These experiments considered in connection with Lusk's observations indicate that the essential physiological peculiarities of gelatin are to be sought in the chemical structure of the *nitrogenous* portion of the molecule; and the failure of the albuminoid to give rise to kynurenic acid in the dog is doubtless associated with the lack of certain aromatic radicals in its make-up. Lastly, attention is directed to the ready assimilation of crystallized vegetable proteid (Dog C), and to the evidence offered of the close physiological relationship between the animal and vegetable products, despite minor differences in chemical structure.[1]

Phlorhizin experiments. — In confirmation of the view that kynurenic acid excretion is incidental to excessive proteid katabolism, additional experiments on animals suffering from phlorhizin diabetes are presented. It has been shown that under these conditions sugar production goes on in the fasting animal directly at the expense of tissue proteid, the amount of nitrogen in the urine going parallel with the amount of sugar excreted;[2] and the sugar excretion may thus go on even in the absence of glycogen in the liver.[3] Lusk[4] and his co-workers have shown that in the fasting dog a constant ratio of dextrose to nitrogen excreted is maintained during phlorhizin diabetes (D: N:: 3.75: 1), and that feeding meat or gelatin does not change the ratio. The figures indicate a production of about 60 grams of dextrose from 100 grams of proteid, and it has furthermore been found that in this form of diabetes the proteid metabolism may increase to an extent as high as 560 per cent above that in simple inanition. Tables J and K show the extent of kynurenic acid production observed on two fasting dogs during phlorhizin diabetes. The phlorhizin, dissolved in dilute sodium carbonate solution, was introduced subcutaneously about every eight hours. Water was given ad libitum.

[1] Cf. RUTGERS: Zeitschrift für Biologie, 1888, xxiv, p. 351.

[2] Cf. for example, CREMER u. RITTER: Zeitschrift für Biologie, 1893, xxix, p. 256.

[3] THIEL: Arch. f. exper. Pathol. and Pharmakol, 1887, xxiii, p. 142. Cf. also HÉDON: Comptes rendus de la soc. de biologie, Paris, 1897, (10), iv, p. 60.

[4] REILLY, NOLAN and LUSK: This journal, 1898, i, p. 395.

DOG K.

Date.	Body Weight.	Urine.						Food.
1898.	kilos	Vol. c.c.	Sp. Gr.	Reaction. litmus	Nitrogen. grams	Kynurenic Acid. grams	Dextrose. grams	grams
May 19	12.4	....	....	....	4.21	0.178	....	none; water.
20	12.3	244	1042	Acid	4.21	0.178	....	" "
21	12.2	....	....	....	2.42	0.065	....	" "
22	12.0	....	....	....	2.42	0.065	....	" "
23	11.9	....	....	....	2.42	0.065	....	" "
24	11.8	290	1046	Acid	2.42	0.065	....	" "
25	11.8	290	1058	"	9.00	0.208	39.15	**phlorhizin.** 1; three times a day.
26	11.6	420	1055	"	8.82	0.255	29.82	" 1; twice a day.
27	....	520	1048	"	10.86	0.342	35.88	none; water.
28	9.9	325	1051	"	7.35	0.172	22.42	" "
29	9.6	210	1050	"	....[1]	0.073	11.55	" "
30	9.4	265	?	"	4.38	0.053	12.98	" "
31	9.2	405	1025	"	2.47	none	....	" "
June 1	9.3	180	1020	"	....	none	....	" "

[1] Fæces in the urine.

Here again the production of kynurenic acid during fasting is observed. Coincident with the appearance of the sugar in the urine occurs a rise in nitrogen excretion accompanied likewise by an increase in kynurenic acid. The figures become more striking when the daily averages of the period preceding and succeeding the phlorhizin days are presented in contrast.

The increased output of kynurenic acid attending the large increase in proteid katabolism (over that in inanition) thus justifies the emphasis placed upon the close relationship between these factors.

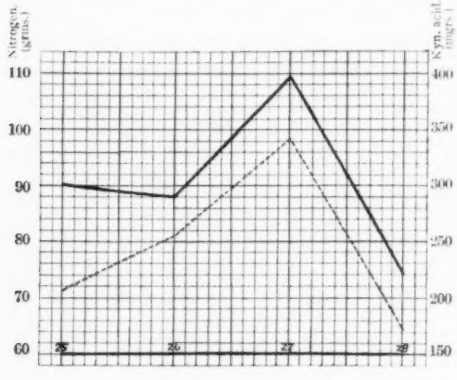

FIGURE 1.

DOG K. The abscissæ represent the successive days of the experiment; the ordinates of the unbroken line represent grams of N excreted, those of the broken line represent milligrams of kynurenic acid.

Owing to less careful conditions of experiment (lack of catheterization, etc.) the ratio of dextrose to nitrogen is somewhat lower than that found by Lusk. In the experiment on Dog K the ratio is higher. In this animal sugar persisted in the urine for several days after the phlorhizin injection. The ratio of dextrose to nitrogen during the phlorhizin days was as 3.68 : 1. The other phenomena observed resemble those of the preceding experiment, namely, increased kynurenic acid excretion accompanying stimulated proteid decomposition,[1] as shown in the curves of Fig. 1.

Amyl nitrite experiment. — The production of diabetes in the dog by means of amyl nitrite has been demonstrated by a number of investigators.[2] We have also studied the action of this drug in the case of a dog. The 10-kilo animal had been fed three days on a diet consisting of albumin, 15 grams; carbohydrate, 50 grams; fat, 40 grams; — under which conditions nothing more than traces of

[1] In the case of one dog which excreted no kynurenic acid during six days' starvation we were unable to get similar results with phlorhizin.

[2] ARAKI: Zeitschr. f. physiol. Chemie, 1891, xv, p. 553. The older literature is referred to here.

kynurenic acid were found in the urine. On the fourth day the animal received three subcutaneous injections of amyl nitrite (about 6 c.c. being given in all). By the following day the dog was dead. The urine (128 c.c.) removed from the bladder contained no kynurenic acid; 4.69 grams sugar were present, and the ratio of dextrose to nitrogen was found as 7.4 : 1. Traces of albumin were also found, in agreement with observations of Araki.[1] The high ratio of dextrose to nitrogen indicates that the sugar found could not have its origin wholly in proteid decomposition, nor was this to be expected under these conditions (previous feeding, etc.). But Thiel[2] has shown that amyl nitrite fails to produce characteristic glycosuria in hens, although phlorhizin is effective in these animals. Amyl nitrite diabetes is therefore doubtless of a distinctly different type from that produced by phlorhizin; at any rate the absence of kynurenic acid in the urine in our single experiment corresponds with the slight evidence of proteid katabolism obtained.

Experiments on other animals. — So far as we are aware, no one has succeeded in finding kynurenic acid in the urine of any animal other than the dog. Hofmeister,[3] after careful examination, failed to find it in human urine. It has likewise been found missing in the urine of the rabbit,[4] wolf,[5] and fox.[5] We have searched for kynurenic acid in the urine of the cat during inanition as well as during meat and milk diet, but always with negative results. In view of the evidence of our experiments regarding the formation of kynurenic acid in the dog from body proteids during inanition, several rabbits were starved for varying periods (three to five days) and the urine then examined. No kynurenic acid was found. Lastly the urine of man has been examined in wasting diseases (severe diabetes) without avail. Since Hauser[6] and Solomin[7] have observed that kynurenic acid introduced

[1] Araki: Zeitschr. f. physiol. Chemie, 1891, xv, p. 553. The older literature is referred to here.

[2] Thiel: Arch. f. exper. Pathol. u. Pharmakol., 1887, xxiii, p. 142.

[3] Hofmeister: Zeitschr. f. physiol. Chemie, 1881, v, p. 69. Eckhard, long before, had missed it in human urine; see Ann. Chem. Pharm., 1856, xcvii, p. 358.

[4] Schmidt: Ueber das Verhalten einiger Chinolinderivate im Thierkörper mit Rücksicht auf die Bildung von Kynurensäure. Inaugural-Dissertation, Königsberg, 1884.

[5] Capaldi: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 87.

[6] Hauser: Arch. f. exper. Pathol. u. Pharmakol., 1895, xxxvi, p. 1.

[7] Solomin: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 497.

into the organism of man, rabbit, or dog is in good part destroyed (especially in the case of the first two), it may be, as Solomin suggests, that kynurenic acid is absent from human and rabbit's urine not because it fails to be produced, but rather because it is destroyed in these organisms as rapidly as it is formed.

Theoretical considerations regarding the quantity of kynurenic acid obtainable. — That the absolute amount of kynurenic acid produced at any time should be large is scarcely to be expected. If, as the experiments of Lusk and his co-workers have indicated, the proteid molecule yields on cleavage in the body an amount of sugar equal to nearly 60 per cent, there remains "a nitrogen-containing radical in which the carbon and nitrogen would appear in the atomic ratio of 2.2 of C to 1 of N."[1] Now kynurenic acid, $C_{10}H_7NO_3$, contains 10 of C to 1 of N; obviously the nitrogenous proteid radicals could not yield large quantities of a body of the composition indicated.

Summary. — Kynurenic acid is a direct product of proteid katabolism, and, as Baumann's experiments indicated, does not owe its immediate origin to putrefactive changes in the intestine.

Kynurenic acid excretion accompanies accelerated proteid decomposition, whether this condition be brought about by starvation, ingestion of large amounts of proteid food, or through the action of drugs (borax, phlorhizin).

Similar results follow the ingestion of both animal and vegetable proteids, as well as proteoses; gelatin, however, does not give rise to kynurenic acid in metabolism, acting precisely like the carbohydrates in this respect. In conditions of ordinary nitrogenous equilibrium or under the influence of proteid-sparing foods, kynurenic acid excretion is greatly diminished or absent.

The observations suggest the presence of quinoline-like radicals in the proteid molecule; the existence of a large carbohydrate group is also confirmed.

Uric acid and kynurenic acid may occur together in dog's urine, as Solomin has found. Kynurenic acid is absent from the urine of the cat during fasting and proteid feeding, and is not found in the urine of the rabbit during inanition.

[1] Reilly, Nolan and Lusk: This journal, 1898, i, p. 409.

ON THE MODIFICATION OF RIGOR MORTIS RESULTING FROM PREVIOUS FATIGUE OF THE MUSCLE, IN COLD-BLOODED ANIMALS.

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THE effect of previous fatigue upon the process of rigor mortis was first investigated by Brown-Séquard.[1] He stated that when one leg of a rabbit was removed immediately after death and stimulated with a constant current, it stiffened in ten minutes, whereas the other leg did not enter into rigor for five hours. Nagel[2] obtained a similar result in the isolated muscle of cold-blooded animals. He fatigued one gastrocnemius of a frog either through strychnia poisoning, or through electrical stimulation of the sciatic nerve for fifteen minutes. A record was taken by the graphic method of the process of rigor in both muscles, and it was found that the tetanized muscle entered into rigor about sixteen hours sooner than the normal muscle; also that the whole amount of shortening in the fatigued muscle was about one-third less than it was in the muscle which had not been fatigued.

Both Brown-Séquard and Nagel obtained their results in the course of experiments undertaken with the object of determining the influence of the central nervous system upon rigor mortis. They did not, apparently, consider the relation of the results they obtained to the question of the direct influence of the previous condition of the muscle. The present investigation was undertaken in order to determine whether the effect of fatigue upon rigor mortis is constant; and this point having been decided in the affirmative, the experiments were continued with the object of ascertaining, if possible, to what changes in the fatigued muscle itself the modification induced by fatigue is due.

[1] BROWN-SÉQUARD: Gazette medicale de Paris, 1849, pp. 881, 999; quoted by M. BIERFREUND: Archiv. f. d. ges. Physiol., 1888, xliii, p. 211.

[2] NAGEL, W.: Experimentelle Unterschungen über die Todten-Starre bei Kaltblütern. Archiv. f. d. ges. Physiol., 1894, lviii, p. 279.

I. Description of Method.

The experiments were conducted entirely upon frogs. Most of the observations were made upon heat rigor, from motives of convenience, although in order to establish beyond doubt that the results obtained in this way could justifiably be applied to the normal process a few experiments were performed in which rigor was allowed to come on in the usual manner. In every instance the results were recorded by the graphic method. The brain and spinal cord of the frog were first destroyed. After this the tendons of both gastrocnemii were freed beneath the skin, and both sciatics were exposed. The frog was now fastened on a board and a hook passed through one of the tendons by means of which the muscle was connected with a lever that recorded the curve of fatigue upon a revolving drum. This lever carried a weight that varied from five to fifteen grams, according to the size of the frog. The muscle was fatigued by stimulation of the sciatic nerve with an ordinary induction coil. Simple breaking shocks were thrown into the muscle at intervals of four seconds by means of a Baltzar drum. It was occasionally found desirable to increase this rate of stimulation when it could be done without inducing symptoms of tetanus. During the stimulation the secondary coil of the induction apparatus was at first placed as far from the primary coil as was possible consistently with the production of maximal contractions; but after symptoms of exhaustion appeared with the coil in this position it was brought gradually nearer until finally complete fatigue with discontinuous stimuli was obtained with the secondary coil over the primary. One Edison-Lalande cell was used in the primary circuit. The time required for complete fatigue varied from one to four and a half hours; during this period the nerve was kept covered with a tent of filter paper wet with normal saline solution, and was also moistened from time to time with the same liquid. When the muscle failed to give any further response to stimulation the frog was taken down and both gastrocnemii muscles removed, the kneejoint and a portion of the femur remaining attached to the muscle. Each gastrocnemius was now placed in an apparatus especially adapted for this experiment. It consisted of a cylinder about three and a half centimetres in diameter, within which was a second cylinder of the same length but much narrower. The lower end of the outer cylinder was closed by a cork, into the middle of which the inner cylinder fitted, and the space between the two was

filled with water. A glass tube, open at one end and sealed at the other, was inserted by its open end into the cork with which the outer cylinder was closed. This tube was twenty centimetres in length, and was bent upon itself so that it projected from the apparatus at an obtuse angle. As the tube communicated by its open upper end with the water-filled compartment between the two cylinders, it was, of course, itself filled with water; and when a Bunsen burner was placed under the closed lower end the heat was transmitted by convection currents to the volume of water above. The inner cylinder mentioned before was closed at its lower end by a rubber stopper, through the middle of which passed a narrow glass tube, and its top was covered by a closely fitting hard rubber cover. When a few cubic centimetres of water were placed in the bottom of this cylinder it became a perfect moist chamber heated by the surrounding water; the temperature within it was recorded by a thermometer which passed through the cover. In this moist chamber the muscle was suspended, the femur to which it remained attached being screwed into a socket in the cover specially intended for this purpose. To the hook inserted in the tendon of the muscle was attached a thread that passed to the exterior through the glass tube in the lower end of the moist chamber. This string was connected with a light aluminium lever carrying a weight of one gram, an amount just sufficient to keep the lever steady. When the two muscles were securely in place, each in its own moist chamber, the two pens at the ends of the levers were adjusted so as to write one above another on a drum revolving at the rate of once an hour. With care and close attention it was found possible to elevate the temperature of the two moist chambers with very nearly the same rapidity. The application of heat by means of the Bunsen burner was continued not only up to the point at which rigor appeared, but afterwards, until the muscle gave no further sign of contraction.

II. Effect of Fatigue upon Rigor Mortis.

When the two muscles, one of which had been fatigued, were heated the following results were obtained. The heat rigor appeared from 5° to 13° C. earlier in the fatigued muscle, the majority of cases showing a difference of not less than 10° C. The rigor was usually complete at nearly the same temperature in both muscles. The whole amount of shortening in the fatigued muscle was one

half to one third less than in the normal muscle. These differences manifest themselves very plainly in a comparison of the tracings taken from the two muscles. An example is given in the accompanying illustration of curves plotted from one of the records.

The results of twelve experiments are given below in tabular form. Four others showed the same general characteristics, although the records were not sufficiently accurate for tabulation. It may be added that several experiments of the same kind performed by the writer in the physiological laboratory of Bryn Mawr College before the present investigation was begun, agree entirely with the later results.

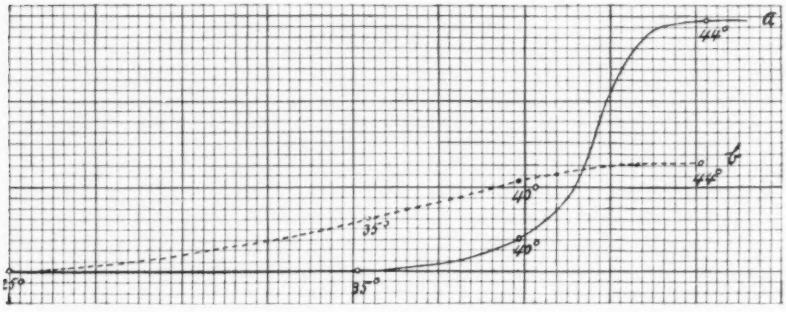

FIGURE 1. One half the original size. Curve of heat rigor in: *a*, the normal muscle; *b*, the fatigued muscle.

In order to determine whether the inferences drawn from the effect of fatigue upon heat rigor could justifiably be applied to rigor occurring in the usual manner, an experiment was performed in which after one gastrocnemius muscle was completely fatigued rigor was allowed to come on in both muscles at the temperature of the room, which was 20° to 23° C. In the normal muscle rigor began in about twenty-two hours, and was complete in about fifty-one hours; in the fatigued muscle it made its appearance in one hour and was complete in six hours. The whole amount of shortening in the fatigued muscle was one third less than in the other. This result coincides exactly with those obtained by the application of heat, and was confirmed by several other experiments of a similar character.

TABLE I.

Showing effect of previous fatigue on heat rigor.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.
I	Normal	38	43	29
	Fatigued	25	41	14
II	Normal	37	44	32
	Fatigued	25	35	11
III	Normal	37	45	29
	Fatigued	25	40	8
IV	Normal	37	43	35
	Fatigued	27	41	20
V	Normal	39	43	25
	Fatigued	34	40	10
VI	Normal	37	42	31
	Fatigued	28	40	10
VII	Normal	37	43	20
	Fatigued	25	40	14
VIII	Normal	38	44	21
	Fatigued	25	30	9
IX	Normal	40	43	28
	Fatigued	30	44	18
X	Normal	39	43	36
	Fatigued	33	40	14
XI	Normal	36	44	38
	Fatigued	27	43	17
XII	Normal	37	43	33
	Fatigued	29	40	19

III. Investigation into the Cause of the Effect of Fatigue on Rigor Mortis.

It having been shown that previous fatigue of the muscle undoubtedly hastened the appearance of rigor and also lessened the extent of shortening, the next step was to investigate the cause of this modification. The first idea that suggested itself was obviously that the removal of the products of fatigue by irrigation with a neutral solution would probably restore the normal rigor.

Irrigation with 0.5 per cent sodium chloride solution. — In this and similar experiments the unfatigued muscle was first excluded from

the circulation by a ligature tied tightly around the thigh. The other muscle was then irrigated by placing a cannula in the aortic arch of one side and ligating all other branches. Thus the entire animal was irrigated with the exception of the head and lungs and the excluded leg. The success of the irrigation in the gastrocnemius muscle was usually indicated by the amount of œdema exhibited. From five hundred to a thousand cubic centimetres of liquid were used at a time.

Four experiments in which the fatigued leg was washed out with normal salt solution for a period of time varying from fifteen minutes to two hours showed no restoration of the normal rigor. Two other experiments in which Ringer's solution (NaCl, 0.7%, 100 c.c.; KCl, 1%, 3 c.c.; $CaCl_2$, 1%, 2.6 c.c.) was substituted for the saline solution, gave the same result. In one of the latter the method was slightly varied by fatiguing both legs and then irrigating one of them, the other fatigued leg serving in this case as a control.

TABLE II.

Irrigation after fatigue with solution of 0.5 per cent NaCl, or with Ringer's solution.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Normal	36	43	21	
	Fatigue + Irrigation .	30	40	9	
II	Normal	34	43	34	
	Fatigue + Irrigation .	29	45	26	
III	Normal	35	41	37	
	Fatigue + Irrigation .	26	39	23	
IV	Normal	37	44	58	
	Fatigue + Irrigation .	27	43	33	
V	Normal	40	42	59	Ringer's solution.
	Fatigue + Irrigation .	32	42	43	
VI	Fatigue	27	41	22	Ringer's solution.
	Fatigue + Irrigation .	28	42	22	Both legs fatigued; one leg irrigated.

Irrigation with sodium carbonate. — As the appearance of fatigue in worked muscle seems to be connected with the formation of acid products during contraction, it seemed desirable to ascertain whether

the normal rigor could be restored in a fatigued muscle by irrigation with a feebly alkaline solution. The muscle was therefore washed out with normal saline solution containing 5 milligrams of Na_2CO_3 to 100 cubic centimetres of liquid. It may be said at once that these experiments proved the least satisfactory portion of this investigation. The difficulty of washing out the blood-vessels with an alkaline solution is very great, for the alkali has a strong tendency to constrict them, so much so that it was sometimes impossible to induce the circulating fluid to flow at all after the passage of 200 to 300 cubic centimetres, even under very high pressure. In all experiments of this nature, therefore, there exists some uncertainty as to whether the fatigued muscle was, or was not, completely washed out. In seven out of eight experiments in which the normal muscle was compared with the one which had been fatigued and irrigated, the curve of rigor in the fatigued muscle retained the characteristics of fatigue, although in one case — number two in the accompanying table — the amount of shortening was very nearly the same in both muscles. In the remaining experiment, number eight, there seems to be a partial restoration of the normal curve; but it is possible that in this case the apparent restoration may be the result of incomplete fatigue of the muscle. Two additional experiments are given in which both muscles were fatigued and one of them irrigated. In one of these, number nine, the irrigation had, practically, no results. In the other, number ten, the muscle was irrigated with Ringer's solution, to which was added the usual percentage of sodium carbonate. This was done in hopes that the potassium salts contained in it might by their relaxing effect upon the blood-vessels counteract the opposite influence of the alkali. This hypothesis proved correct, for eight hundred cubic centimetres of the solution were circulated through the leg without difficulty. The result showed a very slight increase in the amount of shortening in the irrigated muscle, but there was no other evidence of restoration, and the two curves were practically the same.

Irrigation with acids. — As the results obtained by irrigation with an alkali were not entirely conclusive, owing to the difficulty of irrigation, it was decided to attack the question from the other end, and ascertain whether washing out a normal muscle with an acid solution would induce the modification of rigor which occurs after fatigue. One leg was therefore ligated and the gastrocnemius muscle of the other side irrigated with sodium chloride solution containing

TABLE III.

Irrigation after fatigue with normal saline solution containing 0.05 per cent Na_2CO_3.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.
I	Normal	35	42	39
	Fatigue + Irrigation .	26	41	29
II	Normal	37	44	30
	Fatigue + Irrigation .	28	42	28
III	Normal	38	42	39
	Fatigue + Irrigation .	28	42	27
IV	Normal	32	44	27
	Fatigue + Irrigation .	28	40	6
V	Normal	37	42	50
	Fatigue + Irrigation .	25	40	32
VI	Normal	29	40	59
	Fatigue + Irrigation .	26	36	13
VII	Normal	37	43	54
	Fatigue + Irrigation .	26	42	27
VIII	Normal	38	43	27
	Fatigue + Irrigation .	35	42	22
IX	Fatigue	25	42	24
	Fatigue + Irrigation .	27	42	25
X	Fatigue	28	42	23
	Fatigue + Irrigation .	28	43	29

0.025 per cent lactic acid. Two experiments of this kind showed that rigor in the irrigated muscle not only did not set in earlier than in the other muscle, but that its appearance was actually delayed. There was no difference in the amount of shortening in the two muscles. Two experiments in which acetic acid was substituted for lactic gave the same result. As these four experiments are quite positive it seems justifiable to conclude that the effect of fatigue on rigor is not due to the influence of increased acid production during contraction.

Irrigation with carbon dioxide. — One experiment in which the muscle was irrigated for thirty minutes with saline solution saturated with carbon dioxide gave absolutely negative results.

TABLE IV.

Irrigation of normal muscle with normal saline solution containing acid.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Normal	36	43	27	Lactic acid 0.025
	Irrigated	39	45	26	
II	Normal	33	44	26	" " "
	Irrigated	38	44	26	
III	Normal	36	43	17	Acetic acid 0.025
	Irrigated	39	44	16	
IV	Normal	36	43	21	Acetic acid 0.01
	Irrigated	41	44	20	

Irrigation with sodium oxalate. — In order to ascertain whether the variation in heat rigor was due to a possible variation in the calcium salts during fatigue, the muscle was irrigated with saline solution containing 0.4 per cent of sodium oxalate. Three experiments of this kind showed that the rigor of the irrigated muscle was wholly unaffected, although the precipitation of the calcium salts caused its indirect irritability to disappear completely.

TABLE V.

Irrigation with normal saline solution containing 0.4 per cent sodium oxalate.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Irritability after Irrigation.
I	Normal	37	44	54	Normal.
	Irrigated	38	44	57	Lost.
II	Normal	32	42	47	Normal.
	Irrigated	31	42	43	Lost.
III	Normal	38	43	48	Normal.
	Irrigated	38	43	47	Lost.

Irrigation with extract of fatigued muscle. — It seemed possible that the modification of rigor present in fatigued muscle might be

due to the accumulation of waste products other than carbon dioxide or lactic acid. In order to investigate this question the following experiment was made. The lower extremities of two very large frogs were completely fatigued; all the muscle tissue was at once removed and extracted with seventy-five cubic centimetres of normal saline solution at 45° C. for about thirty minutes. A medium sized frog was placed completely under the influence of curare, after which a ligature was tied around the right leg, and the left leg irrigated with the saline extract at short intervals for about half an hour. This method is precisely similar in its details to that given by Ranke in his work on *Tetanus*, and the passage of the fluid through the blood vessels was accompanied by all the symptoms which Ranke describes, namely, general twitching of the muscles, loss of direct irritability in them, and arrest of the heart-beat. At the end of irrigation the direct irritability of both muscles was carefully tested, and found to be almost completely abolished in the irrigated muscle, although it remained normal in the other. It was confidently expected that after such indications of the effect of the irrigation upon the whole organism some modification of rigor would ensue; on the contrary, it was found that the heat rigor pursued its normal course in both muscles, the only difference being that the amount of shortening in the irrigated muscle was somewhat less than in the other. Two other experiments of a similar nature gave like results, but in both the amount of contraction was greatest in the case of the irrigated muscle. It would seem, therefore, that the variation in this respect may be considered accidental, the character of the two curves being the same in all cases. In one additional experiment an alcoholic extract of fatigued muscle was substituted for the saline extract previously used. This alcoholic extract was prepared by cutting up the fatigued muscles very fine, grinding them in a mortar, and then placing them in five times their own amount of 95 per cent alcohol. After three days the alcohol was filtered off and evaporated to dryness; the residue was then extracted at the boiling point with 200 cubic centimetres of normal saline solution and filtered. The reaction of this extract as well as that made with saline solution was distinctly, though not strongly, acid. The results of this experiment were precisely similar to those just described, except that the symptoms accompanying the passage of the alcoholic extract through the blood-vessels were somewhat less severe than during the circulation of the saline extract.

TABLE VI.

Irrigation with extract of fatigued muscle.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Normal	38	43	64	Saline extract.
	Irrigated	38	44	50	
II	Normal	35	43	37	" "
	Irrigated	35	43	44	
III	Normal	36	43	31	" "
	Irrigated	35	42	35	
IV	Normal	39	42	55	Alcoholic extract.
	Irrigated	38	42	55	

Irrigation with dextrose. — As it seemed clearly established that the changes in rigor mortis after fatigue could not be explained by any one of the hypotheses that at first seemed reasonable, the idea now suggested itself that possibly the using up of the glycogen which is known to occur when muscle is severely worked might be responsible for the alteration in the rigor. An effort was therefore

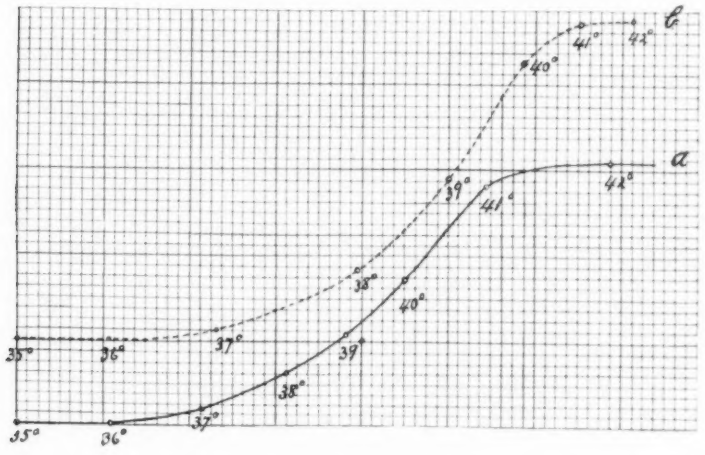

FIGURE 2. One half the original size. Curve of heat rigor in: *a*, the normal muscle; *b*, the fatigued muscle irrigated with dextrose.

made to restore the deficient glycogen by supplying the muscle with dextrose in the proportion normally present in the blood. The experiments for this purpose were made in three ways.

1. The method first employed was to fatigue one gastrocnemius muscle completely by stimulation of the sciatic nerve, and then to irrigate it for two hours with saline solution containing 0.1 per cent of dextrose. Ten experiments of this nature showed a complete restoration of normal rigor in the fatigued muscle in but three cases. A curve plotted from one of these records is given here. Five of the remaining seven experiments showed a partial recovery; in the remaining two the irrigation produced no effect whatever. It is only fair to say, however, that in one of these failures the saline solution used for irrigation was not fresh, and in the other the irrigation is noted as not satisfactory.

TABLE VII.

Irrigation with dextrose after fatigue; one muscle normal.

No.		Rigor begun. degree	Rigor ended. degree	Amount of shortening in mm.	Remarks.
I	Normal	33	42	59	
	Fatigue + Irrigation .	34	42	51	
II	Normal	36	42	47	Shown in plotted curve.
	Fatigue + Irrigation .	36	43	51	
III	Normal	37	42	39	
	Fatigue + Irrigation .	35	44	24	
IV	Normal	37	44	51	
	Fatigue + Irrigation .	35	42	44	
V	Normal	40	44	28	Saline solution stale.
	Fatigue + Irrigation .	35	42	9	
VI	Normal	39	46	43	Irrigation not satisfactory.
	Fatigue + Irrigation .	32	43	10	
VII	Normal	40	45	32	
	Fatigue + Irrigation .	32	41	24	
VIII	Normal	36	42	51	
	Fatigue + Irrigation .	34	42	42	
IX	Normal	34	42	42	
	Fatigue + Irrigation .	34	43	39	
X	Normal	36	43	51	Ringer's solution.
	Fatigue + Irrigation .	35	41	48	

2. Both muscles were fatigued at the same time by placing the two sciatic plexuses upon the same electrode. One of the muscles was then irrigated with saline solution containing 0.1 per cent of dextrose. In some cases the other leg was simply ligated: in others it was removed from the body and kept wrapped in filter paper moistened with saline solution. Seven experiments carried out in this manner showed recovery in the irrigated muscle to a greater or less extent in all cases. In one experiment the recovery was apparently complete. A curve plotted from this record is given here.

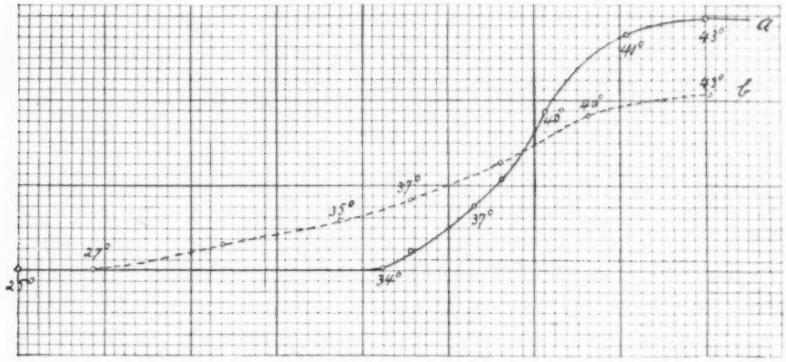

FIGURE 3. One half the original size. Curve of heat rigor in: *b*, the fatigued muscle; *a*, the fatigued muscle irrigated with dextrose.

It will be seen in Table VIII, which gives the results of these experiments, that the period of rest allowed to the fatigued but isolated muscle while the other was undergoing irrigation did not in the least restore the normal rigor.

3. One muscle was fatigued and allowed to go into heat rigor at once, while the other muscle was irrigated with the dextrose while it was being fatigued. Of three such experiments, two showed complete recovery, the third was practically an entire failure.

To supplement these three series an experiment was performed in which after both muscles had been fatigued and one of them irrigated with dextrose they were allowed to go into normal rigor. The fatigued muscle began to contract at once and its rigor was complete in about seven hours; the muscle that had been fatigued and irrigated after fatigue, showed no signs of rigor until the tenth hour, and

TABLE VIII.

Irrigation with dextrose after fatigue; both muscles fatigued.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.	Remarks.
I	Fatigue	27	42	38	
	Fatigue + Irrigation .	33	42	42	
II	Fatigue	26	42	35	
	Fatigue + Irrigation .	33	43	47	
III	Fatigue	32	40	31	
	Fatigue + Irrigation .	33	40	45	
IV	Fatigue	31	43	42	
	Fatigue + Irrigation .	36	42	58	
V	Fatigue	30	42	27	
	Fatigue + Irrigation .	30	42	34	
VI	Fatigue	28	44	30	
	Fatigue + Irrigation .	32	43	29	
VII	Fatigue	28	42	39	Shown in plotted curve.
	Fatigue + Irrigation .	36	42	51	

TABLE IX.

Irrigation with dextrose during fatigue; both muscles fatigued.

No.		Rigor begun. degree	Rigor complete. degree	Amount of shortening in mm.
I	Fatigue	32	42	31
	Fatigue + Irrigation .	37	44	50
II	Fatigue	27	40	25
	Fatigue + Irrigation .	39	44	44
III	Fatigue	31	41	42
	Fatigue + Irrigation .	34	44	38

ceased to contract in about twenty-two hours. The amount of contraction in the two muscles was very nearly the same. It should be noted that during this experiment the temperature of the room was unusually high, — 25° to 27° C. This fact probably accounts for the occurrence of rigor in the irrigated muscle as early as the tenth hour,

which is sooner than is usual in normal rigor. The same circumstance may perhaps account for the fact that the amount of shortening was no greater in the irrigated muscle than in the other; for both muscles when they were removed from the apparatus at the end of twenty-three hours showed marked evidence of decomposition, and as rigor in the irrigated muscle was not complete until the end of the twenty-second hour, this condition must have been present during its later stages. Unfortunately there was no opportunity of repeating this experiment at a lower temperature.

One other experiment was performed, in which a fatigued muscle was irrigated in the usual manner with a solution of cane sugar instead of dextrose. This irrigation produced no effect whatever upon the ordinary fatigue curve of heat rigor.

It will be seen from the accompanying tables that there are only three cases in which the variation in heat rigor caused by fatigue has been wholly unaffected by irrigation with dextrose. For two of these cases — numbers five and six in table seven — some explanation can be offered; this leaves only one case of entire failure, — number three in table nine. Thus eighteen out of the twenty-one experiments — more than two-thirds of the whole number — show a restoration of the usual heat rigor, either partial or complete, after irrigation with dextrose. We cannot refer this beneficial action of the dextrose solution to a mere physical change in the liquid, such as increased osmotic pressure, since the experiments with other irrigating fluids make such a conclusion impossible. The sugar probably acts chemically in restoring the normal composition of the muscle substance, and it seems justifiable to infer that the changes in the rigor curve that have been described as the result of fatigue are connected in some way with the using up of the glycogen contents of the muscle.

IV. Conclusions.

1. After prolonged fatigue from electrical stimulation the muscle enters into rigor much earlier, and shortens much less than when not fatigued.

2. This modification of rigor is not due to an increase in lactic acid, or to an accumulation of carbon dioxide, or to the heaping up of other fatigue products, or to the abstraction of calcium salts.

3. It is due to the exhaustion of the glycogen normally present in muscle. For the circulation through the exhausted muscle of a

liquid containing dextrose in the proportion normally present in the blood effects a more or less complete restoration of the normal rigor.

It should be remarked here that it was of course impossible to judge in any experiment when the glycogen contents of the muscle had been restored. To make the experiments comparable it was thought best to use always the same amount (one litre) of the irrigating fluid, and to allow as nearly as possible the same time (two hours) for its passage through the blood-vessels. By this means five complete recoveries in twenty-one experiments have been obtained; and this proportion seems quite as large as could be expected. In the other experiments in which the restoration was only partial, it seems reasonable to conclude that had it been possible to use a more normal circulating liquid, such as frog's serum, in place of the 0.5 per cent saline solution, the restoration would have been more complete.

V. General Observations.

It has sometimes happened in the course of this investigation that results have been obtained which, while they did not bear upon the main point at issue, were not without interest for other reasons. A brief allusion to these observations seems therefore not out of place.

Nervous system. — The influence of the central nervous system upon rigor mortis has been the subject of repeated investigation. Nagel, Bierfreund, von Gendre, Aust, von Eiselberg, all claim to have demonstrated that the central nervous system exerts a marked influence over rigor. Tamassia, on the other hand, believes himself to have shown positive evidence to the contrary. In the course of the present investigation it was found that in the experiments already mentioned, in which the muscle was irrigated with sodium oxalate, the irritability of the muscle was completely lost, although heat rigor occurred in the usual manner. These results, which are given in Table V, agree with Howell's[1] results obtained on normal rigor. Further, it was observed in these experiments that the irritability which was completely lost to indirect stimulation of the muscle after fatigue, was sometimes restored by irrigation with dextrose; quite as often, however, the irrigation was without effect in this particular. This recovery or non-recovery of irritability was wholly independent of the restoration of heat rigor; for it was observed that recovery in

[1] Howell: Journal of physiology, 1894, xvi, p. 482.

this latter respect might be marked in cases where the irritability of the muscle was very slight; and in two instances where a complete restoration of normal heat rigor occurred in an irrigated muscle its irritability was almost absent. So far then as these results go, they agree, not with the majority of observers, who consider that rigor mortis is under the direct control of the central nervous system, but with Tamassia, who states that it has no influence on the progress, the intensity, and the duration of cadaveric rigidity.[1]

Effect of acids and alkalis. — It was mentioned in the early part of this article that irrigation with either lactic or acetic acid caused a delay in the appearance of rigor in the irrigated muscle. On trying the effect of an opposite treatment by washing out a normal muscle with a saline solution containing 0.05 per cent of sodium carbonate, a result exactly the reverse of the former was obtained; rigor appeared in the irrigated muscle at a temperature several degrees lower than in the muscle of the other leg. The extent of shortening was not affected by either acid or alkali. It is well known that the heat coagulation of proteid substances is hastened by the presence of acids and retarded by alkalis; and as the results just mentioned show that these substances affect the condition of heat rigor in a wholly opposite manner, they may be taken as additional evidence in favor of the theory which considers rigor caloris to be a genuine rigor mortis rapidly developed under the influence of the high temperature, and not due to a simple heat coagulation of some of the proteids of the muscle.

Effect of irrigation with dextrose on muscle irritability. — It has been mentioned that in some of these experiments both muscles were fatigued, and one of them irrigated with dextrose while undergoing fatigue. It seemed *a priori* probable that the muscle which was supplied with sugar during its stimulation would require a longer time to become exhausted than the other, which was simply stimulated to the point of fatigue. On the contrary, the muscle irrigated with dextrose became exhausted much sooner than the other; and in one case, where the irrigation was begun fifteen minutes before the stimulation, the irrigated muscle almost immediately refused to respond to stimulation through the nerve. As the stimulation of the muscle in all these cases was effected through the nerve, it may be that the comparatively early loss of irritability in the irrigated muscle was

[1] Tamassia: Rivista sperimentale di frenatria e di medicina legale, 1882, viii; quoted in Archives italiennes de biologie, 1882, ii, p. 254.

owing to some interruption of the connections between the nerve and muscle fibres — for instance, by injury of the end plates in consequence of the œdematous condition provoked. Whether this was the case was not ascertained by experiments on the direct irritability of the muscle in this condition; so that, under the circumstances, it is not advisable to base any conclusions upon this peculiarity. The tendency of these experiments, however, has been to indicate that there is but little parallelism between the conditions necessary for normal contraction and those required for normal rigor. That the controlling conditions of the two phenomena are not identical is indicated also by other facts which have been referred to briefly in this paper. Thus it has been shown that when a muscle is irrigated with sodium oxalate it loses its irritability to direct as well as indirect stimulation, but its heat rigor comes on at a normal temperature and follows apparently a normal curve. It would appear from this that although calcium salts in precipitable form are necessary to the normal contraction, they play no part in the contraction of rigor mortis. Again, irrigation of a normal muscle with a saline extract of fatigued muscle diminishes or destroys its irritability to direct stimulation, but the rigor curve of such a muscle preserves practically its normal characteristics, — that is, it suffers no diminution in amount and begins at the normal temperature. This fact indicates that although the waste-products of muscle contraction — so-called fatigue substances — either diminish or entirely suppress the normal contraction, they have no perceptible influence on the changes leading to the rigor contraction, the modification of this last phenomenon following on muscle work being due, as this paper is intended to show, to changes in the glycogen-contents of the muscle.

I wish, in concluding, to express my strong sense of obligation to Professor Howell, under whose direction this investigation has been carried on, for his very kind interest both in the experimental work and in the preparation of this paper for publication.

I am also indebted to Professor Warren of Bryn Mawr College, in whose laboratory the initial experiments were made, and who first suggested to me that I should investigate the influence of fatigue on rigor mortis.

ON THE RELATION OF THE BLOOD TO THE AUTOMATICITY AND SEQUENCE OF THE HEART-BEAT.

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INTRODUCTION.

THE nature of the inner stimulus that arouses the heart contractions is so hidden in obscurity that but few attempts have been made to explain its probable origin, although it may well be supposed that few problems in physiology have offered such inducements to investigation and speculation. To the specialist the problem resolves itself into a study of the metabolism of the heart tissues, and it is needless to say that our knowledge of the chemical structure of the tissues involved and their reactions during metabolism is so great as to restrain effectually all legitimate speculation to very modest limits. Although a final explanation of the cause of the heart-beat seems clearly impossible at present, yet no physiologist perhaps doubts that it will be attained eventually by the persistent use of methods such as are being employed to-day; and every contribution, however incomplete in its results, it may be hoped will add something to the better understanding of the conditions and difficulties of the problem.

For many years indeed the conditions controlling the heart-beat have been studied and analyzed with care by numerous observers, with the result that certain preliminary views with regard to its causation have become more or less accepted in physiology. Some of these ideas, especially in the matter of the relation of the constituents of the blood to the heart-beat, have seemed to the author to be erroneous, and the present paper is written mainly to correct, if possible, certain misconceptions regarding the part taken by the blood, and to point out what appears to be an important, although somewhat neglected factor, in the production of the inner stimulus through which the so-called automatic contractions are initiated. This paper and the accompanying one by Dr. Greene may be considered as a continuation of the work begun by the author some years ago.

It may be accepted without discussion that at each contraction of the heart muscle, as in the case of ordinary striped muscle, there is a

chemical change attended by a liberation of energy that formerly existed in potential form. This chemical reaction we can suppose may be initiated by some form of energy falling into the substance of the heart muscle from without, by a nerve impulse, for example; or it may be purely spontaneous or automatic, arising from the intra-molecular movements of some highly unstable substance; or it may follow directly or indirectly from a definite chemical reaction between an energy-containing substance within the heart and some other substance or substances formed periodically within the heart or already existing in the liquids of its tissues. Haller[1] traced the origin of the stimulus to an action of the blood, and in the early part of this century this idea was still prevalent even after the discovery of the existence of nerve cells in the heart. Thus J. Müller[2] speaks of the stimulus as coming from a "constant mutual action which is going on between the blood in the capillaries or between the cardiac nerves and the texture of the heart." Budge[3] supposes that a constant stimulus is in action, coming to the muscle through its motor nerves, but originating in the latter through the action of the blood and not automatically.

Later the source of the inner stimulus seems to have been referred generally to the nerve cells in the heart without specific theories as to its mode of origin. Langendorff,[4] however, makes the specific assumption that the immediate cause of the heart's contractions is to be found in the automaticity of the intrinsic ganglia, and that within these nerve cells the impulse arises as the result of dissociation processes that take place in the heart itself. "Das Lebensproduct der Zelle ist ihr Erreger." According to Langendorff the blood is essential as a nutrient liquid, but possesses no stimulating action. It forms therefore an essential condition for the action of the inner stimulus without itself causing this stimulus directly. Engelmann[5] adopts a similar hypothesis, in that he suggests that there is a continual production of a stimulus within the heart muscle owing probably to the fact that the metabolism of the muscle itself liberates substances which act as stimuli. He further supposes that the continuous stimulus thus provided for, is converted into a periodic

[1] "Qui hos experimentorum nostrorum eventus pensitaverit, is quidem non dubitabit nobiscum pronunciare, causam, quae cor in motum ciet, omnino sanguinem venosum esse. Nam enata ea causa cor movetur, subtracta quiescit, diminuta motus cordis languet, aucta motus intenditur." (See No. 1 in the list of references at the end of the paper.)

stimulus by the fact that these substances are temporarily antagonized or destroyed by other products formed during systole or by a reaction developed during systole. According to this hypothesis it would seem that the substances acting as a stimulus arise mainly from the metabolism of the heart muscle during its period of rest.

Recent authors have agreed quite unanimously that it is useless to attempt to refer the origin of the inner stimulus to the blood or any other agency external to the heart muscle itself or its nerve cells. This conclusion the present author has questioned in a previous paper.[6] Influenced by the important researches of Ringer[7] as well as by results of my own, I have long been convinced that the inorganic salts of the blood and the liquids of the heart tissue, and especially the calcium compounds, stand in a peculiar and fundamental relation to the initiation of the inner stimulus of the heart contractions. The general idea here expressed is not new. In the interesting series of researches from the Leipzig laboratory bearing upon the relation of the inorganic salts of the blood to the heart-beat, this view is implied or stated in several instances, particularly in the paper by Merunowicz.[8] According to this author the automatic contractions of the heart depend upon both the organic and the inorganic constituents of the surrounding liquid. The former furnishes the supply of nourishment from which the energy of the contraction is obtained, while the mineral constituents give to these compounds such a form as enables them to be used within the muscle in the production of a contraction.

Merunowicz and those who immediately followed him were in ignorance of the specific influence of calcium compounds upon the heart's contractility. The discovery of this important fact we owe to Ringer.[7] As the result of his work especially it becomes necessary to consider the inorganic as well as the organic constituents of the blood, and the familiar arguments intended to show that the blood or lymph does not contain substances capable of initiating the stimulus to the heart-beat, seem to the author to fail completely, for, in this sense, no one has shown that a bloodless heart is capable of beating. If, as the author believes, the origin of the inner stimulus is to be traced to the action of certain inorganic constituents in the liquid bathing the heart tissue, it becomes necessary first to prove the efficiency of these constituents in maintaining the heart-beat in solutions from which the organic constituents of the blood are excluded. This is particularly important because of the peculiar views advo-

cated by Kronecker and his pupils in a number of papers.[9] In these researches Kronecker, or those working under his direction, have attempted to demonstrate that the energy of the heart contraction is derived immediately from the serum-albumin of the blood (or lymph) bathing the heart tissue. In the earlier papers of this series the authors were led to erroneous conclusions because of their ignorance of the specific importance of the calcium compounds. For instance, Kronecker[9] believed that he had proved that peptone, which alone or in solution in physiological saline will not maintain contractions of the heart muscle, is rendered capable of performing this function when allowed to remain a short time in the stomach or intestine of a living animal. This result he explained on the assumption that the peptone is thereby converted to serum-albumin, although he furnished no chemical proof that such a transformation occurs. While it is possible that under the given conditions peptone is transformed to some other proteid, and perhaps to something having peculiar nutritive relations to the heart, still it must be borne in mind that under these conditions the solution becomes mixed with the inorganic salts of the gastric or intestinal secretion, peptone, in the case of the gastric juice at least, being an efficient secretogogue. These salts alone, as has been shown,[6] may have been sufficient to give the result observed, and their action should be considered before any assumptions are made as to the conversion of peptone to serum-albumin and a specific effect of the latter on the heart.

So also with regard to the claim made by Martius[10] that a heart irrigated successively with sodium chloride 0.6 per cent and sodium chloride 0.6 per cent made alkaline with sodium carbonate, is brought into a condition in which nothing but a liquid containing serum-albumin can make it beat; for the error of this statement was easily shown after Ringer had called attention to the effect of calcium salts. In the latest paper by White[11] the wonderful efficiency of the calcium salts mixed in certain proportions with potassium and sodium salts is duly recognized, but the contention is still made that the presence of serum-albumin in the liquids of the heart is always an immediate necessity for the production of the heart contractions, these contractions ceasing so soon as the serum-albumin is completely removed. The fact that the ventricle of the frog's heart may continue to beat after steady irrigation for many hours with physiological saline, Martius's liquid and Ringer's mixture of salts, is explained upon the assumption that the heart-beat is maintained during this time by

minute residues of blood, with its contained serum-albumin, that have been captured in the capillary crevices of the spongy heart tissue. The point is clearly made in these papers that the heart contains no supply of stored energy in its own substance available for contraction, but derives the energy for contractions directly from the serum-albumin of the blood. In default of the presence of this constituent, contractions of the heart muscle must cease, no matter what other conditions may prevail.

The present author, on the contrary, has been compelled by his experiments to adopt the view of Gaule,[12] namely, that the energy of the heart-beat is derived from material stored in its own substance, material which in the case of the isolated heart supplied with an "inorganic diet" is gradually consumed. For the utilization of this supply of energy, however, certain conditions are necessary, and the principal one of these conditions seems to be the presence in the liquids of the heart of a supply of calcium in some form. In what way the calcium compounds are concerned in the katabolism of the energy-yielding substance in the heart need not be discussed now, nor the antagonistic influence of the potassium compounds. Strong evidence, however, may be presented to show that the calcium compounds have this specific function, and may therefore be regarded in a sense as the source of the so-called inner stimulus.

When Kronecker assumes that a ventricle or a strip of heart muscle, that has been irrigated or bathed for thirty to fifty hours in large quantities of a liquid containing only inorganic salts, continues to beat by virtue of the serum-albumin present in the capillary spaces of the tissue, he makes an assumption that can neither be proved nor disproved positively. By virtue of his hypothesis, traces of serum-albumin far too minute to be detected by the chemical means at our disposal may continue to supply energy for contractions; if, therefore, so long as contractions continue he claims that serum-albumin is present, no one perhaps can definitely prove the contrary. In judging this matter, therefore, we should consider the probabilities in the case, and should adopt the explanation that is the simplest and most direct conclusion from the experimental evidence. It must be remembered in this connection that at no time has any direct evidence been given that serum-albumin is used in or is immediately necessary to the heart contractions. The most that has been done is to show that liquids containing serum-albumin such as lymph or blood (or milk) will best support the contractions of the

isolated heart. Considering the complex composition of these liquids, it is somewhat gratuitous to assume without further proof that their favorable action on the heart is owing to the presence of serum-albumin.

It is necessary here to distinguish also between the nutrient action of such liquids and a possible stimulating action. On the assumption that the heart contains a supply of organic material capable of yielding by katabolic changes the energy for contraction, it is perfectly evident that this supply will be consumed in an isolated heart after a shorter or longer interval, and that for further continuance of the power of contraction new material must be formed within the heart by metabolic processes. That the blood and lymph contain substances for the nutrition of the heart and the building up of its energy-yielding compounds, must be admitted on general grounds, as in the case of ordinary striped muscle or other active tissues. It is highly probable, moreover, that this nutritive material consists in part, at least, of proteid compounds; but that this nutritive supply is to be found especially in the serum-albumin of the blood is an entirely unproved hypothesis, although one very commonly made. But granting that this may be the case, that the serum-albumin and possibly the other proteids of the blood furnish the raw material for nutrition, this is quite a different matter from the hypothesis made by Kronecker, that the serum-albumin of the blood or lymph is directly utilized by the heart tissue in contraction, and that contraction is impossible if the surrounding liquid does not contain this substance. One may grant, indeed one must grant, that the blood supplies nourishment to the heart; but in addition it is possible that by virtue of some of its constituents it may be concerned in initiating the heart contractions, — in furnishing, in other words, the conditions for the production of the inner stimulus.

As opposed to Kronecker's hypothesis and in support of the view that the immediate cause of the heart contractions is to be connected with the inorganic compounds in the liquids of the heart, and especially with their calcium constituents, attention may be called to a number of facts that have been described by different investigators. It is not claimed that any or all of these facts give indisputable proof of the view here advocated, but so far as the author can judge, this view gives a reasonable explanation of these facts as a whole.

When the whole heart of a frog or terrapin is isolated and is irrigated freely with a continuous supply of saline solution (0.6 to 0.8

per cent NaCl) it ceases to beat within a comparatively short period, varying perhaps from one half to three or four hours. If this solution is then replaced by Martius's liquid, physiological saline made alkaline with sodium carbonate (3 to 5 mgms. to each 100 c.c. of saline) and the irrigation is continued, further contractions may be obtained, the duration of which is also quite variable, the maximum being perhaps about three hours. If then the Martius's liquid is replaced by a Ringer's mixture of calcium, potassium, and sodium salts, the heart will continue to beat for many hours. The precise time that the contractions will keep up on this last solution seems to depend partly on the composition of the mixture itself and partly on the condition of the heart. That the latter factor must influence largely the duration of the contractions follows as a necessary result from the hypothesis that the isolated heart beats on the supply of material stored within its substance, since this supply will vary with the previous nutritive condition of the animal. The very great difference in duration of contraction shown by different hearts under conditions otherwise the same, is quite what we should expect upon this hypothesis. But in addition, it is easy to show that the duration of contractions upon Ringer's mixture is often greatly influenced by the composition of the solution. The usual mixture that has been employed in this laboratory as imitating most closely the composition of the blood, has consisted of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.03%. After an isolated heart has ceased to beat upon this mixture, it often happens that increasing gradually the percentage of $CaCl_2$ will call forth renewed contractions for hours. Upon these successive solutions containing only inorganic salts, the frog's heart will continue to beat in favorable cases for as much as thirty hours, and in the case of the terrapin's heart for even a longer period. It is to be remembered that during this entire period the heart is irrigated freely with the solutions and that liquid that has passed once through the heart is not used again. During such an experiment, therefore, the heart will be irrigated with several litres of solution. Similar results have been obtained upon small strips of muscle from the ventricle of the terrapin, as is reported by Greene in an accompanying paper, and additional results of the same character on the muscular tissue of the venae cavae will be reported in detail below. Now to explain the long-continued contractions on the Ringer's mixture, under the conditions described, on the hypothesis that serum-albumin or any other organic con-

stituent of the blood is still contained in the blood-spaces of the tissue in sufficient quantities to supply the energy for the contractions, seems to the author very improbable, and demands something more than its mere statement to render it acceptable. The more probable view surely is that the liquid irrigating the heart during this period is itself in some direct connection with the causation of the beats, particularly when it is found that the composition of this liquid must be of a definite character.

Additional facts that tend in the same direction are as follows: Aqueous extracts of the thoroughly dried residue of blood, or of milk, containing the inorganic salts and other aqueous extractives, but only scarcely detectable traces of proteid, may support the contractions of the heart of the terrapin for eleven hours, and more, when the heart has been previously washed thoroughly with physiological saline for an hour or more, until the beats have become very feeble.[6] So, too, Merunowicz[8] reports that extracts of the serum, after precipitation with alcohol, will revive the ventricle after it has been exhausted by physiological saline. More conclusive still, if the serum, after precipitation with alcohol, is evaporated to dryness and incinerated, the aqueous extract of the ash, containing presumably no proteid at all, exercises almost as favorable action as the whole serum when used upon a ventricle previously exhausted with normal saline. On the other hand, blood serum, from which the precipitable calcium has been removed by the addition of a slight excess of sodium oxalate, behaves toward the terrapin's heart much like simple solutions (0.6%) of sodium chloride,[5] in spite of the fact that the proteids and other constituents, except the calcium salts, are unchanged. It may be urged against this last experiment that the small excess of sodium oxalate acts as a poison, preventing the normal effect of the other constituents of the serum. This objection is difficult to meet, since it is practically impossible in such a liquid to precipitate exactly the calcium salts without having an excess of oxalate, or, on the other hand, to exactly remove the excess of oxalate without introducing an excess of calcium. That the small excess of oxalate has a specifically injurious action is, however, rendered improbable by the facts that addition of excess of calcium chloride will promptly revive the heart-beats, and, moreover, normal saline solutions (0.6% NaCl), containing about the same excess of oxalate, bring the heart to a standstill only gradually, and after standstill is nearly or quite obtained, the prompt use of a Ring-

er's mixture will revive the heart-beats, as after the use of physiological saline alone. These latter statements are true only for hearts that are in good nutritive condition to begin with, as is the case also when normal saline alone is used. Those who make such experiments will find that some hearts, presumably those in a previously bad condition of nutrition, after being washed to a standstill with sodium chloride 0.7 per cent, do not revive upon Ringer's mixture, and with great difficulty, if at all, upon blood serum. Again, the action of physiological saline upon the heart is not improved by the addition of at least one of the proteids of blood, namely, paraglobulin. This has been shown by Stiénon,[13] as well as by myself.[6] Similar experiments with serum-albumin have not as yet been attempted.

Interesting experiments of the same general character, but made upon strips of the ventricular muscle, are reported in the accompanying paper by Greene. Some of the facts there stated may be repeated here, briefly, to still further emphasize the point we are making. If the heart of a terrapin is thoroughly irrigated with large quantities of sodium chloride 0.7 per cent until the contractions entirely cease, or become very feeble, a process which may require one or two hours of constant irrigation, and if, then, a slender strip from the apical portion of the ventricle is suspended in a mixture of calcium, potassium, and sodium salts in proper proportions, it will again beat with force and regularity for many (50 to 70) hours. It will be found, moreover, that such a strip will not contract rhythmically for long periods, if at all, in solutions of sodium chloride alone, of sodium chloride plus potassium chloride, of sodium chloride, potassium chloride, sodium carbonate, sodium hydrogen phosphate, or with any of these salts with the addition of dextrose. In fact, none of the salts present in the blood will provoke sustained contractions of a heart, or heart strip, previously irrigated thoroughly with physiological salt solution, unless some calcium salt is also present.

Kronecker would explain all the foregoing instances of prolonged sustaining action of calcium-containing liquids upon the heart or the heart strips, by the assumption that the ventricular muscle still contained in its spongy meshwork sufficient blood and serum-albumin to supply the energy for the heart contractions. Under the conditions specified, such an assumption is improbable to begin with, and in the case of the ventricular strips, Greene has shown that it leads to a *reductio ad absurdum*, if the amount of work that the strip performs upon an "inorganic diet" is calculated, and the energy required is

referred solely to serum-albumin, or other proteids that might be present in the blood-spaces of the heart.

It will be seen that in explaining the beat of the isolated heart, fed only with solutions containing inorganic salts, Kronecker is obliged to emphasize greatly the importance of the spongy character of the musculature as affording capillary crevices in which remnants of blood may be captured, and from which they are washed out with great difficulty. It has seemed to me, therefore, that his views might be tested still further by using parts of the heart where this characteristic of the muscular tissue is less strikingly developed. For this reason, in part, I have performed a number of experiments upon muscular strips, taken usually from the right superior cava of the terrapin's heart, at a point near the union of the vein and sinus. The vena cava, at the position selected, shows on its inner surface a slight network of muscular trabeculæ. The network is, however, so slight and superficial that it would seem to be impossible to contend that the shallow spaces thus formed could protect remnants of blood from being washed away after prolonged baths in large volumes of liquid.

Automatic Contractions of Strips of Vena Cava.

The experiments that were made with the strips of vein were of a simple character so far as the technique was concerned. A ring of the vein was cut out at the place selected, and this ring was then opened at one point so as to give a narrow strip of vein with the muscle fibres running longitudinally. This strip was attached to a counterpoised lever so arranged that the weight upon the strip could be changed at will, although as a matter of experience only two weights were used, one giving a load of 500 mgms., and the other a load of 100 mgms. The strip was enclosed in a wide glass tube that could be filled with liquid of any desired composition, and an arrangement was provided to enable the experimenter to draw off the liquid readily from below at any time without disturbing the attachments of the strip. The end of the lever was armed with a delicate strip of thin paper to serve as a writing-point. In this way records of the contractions were obtained upon the blackened surface of a kymographion. Two kymographions were used in the experiments. One of these was especially constructed to make one revolution in something over eleven hours, and was used chiefly to take the records during the night. The other was an endless roll kymographion of

the Hürthle pattern arranged to run at its slowest speed, eight to fifteen millimetres per second. In the experiments this kymographion is designated as the fast drum, and records were taken upon it usually during the day or whenever it was desirable to know the rate of beat. A record of minutes was taken upon both kymographions by means of a specially arranged clock.

The records thus obtained of the contractions of the vein strips were often very striking for the force and regularity of the beats. The relations of these contractions to the composition of the surrounding liquid will be referred to subsequently. At present, as bearing upon Kronecker's hypothesis, I desire to emphasize the length of time such strips may continue to beat in baths composed of solutions of inorganic salts. It may be stated that the inorganic salts used were always purified by several recrystallizations, beginning with salts purchased from the manufacturers as chemically pure. The water used was always distilled from glass. A brief record of those experiments in which the longest duration of contractions was observed will suffice to give the general character of the experiments and the results obtained.

Experiment of March 9 and 10, 1898. Strip of vein from the right superior cava of the terrapin. Strip rinsed in Ringer's mixture and then suspended in large bulk of same and attached to a recording lever. The contractions of the strip were magnified six times by the lever. The load extending the strip was 500 mgms. in the beginning, but later was changed to 100 mgms. The composition of the Ringer's mixture used in the beginning of the experiment was NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.04%. The strip was suspended at 3.30 P. M., March 9. Began to beat at once, showing also rhythmic tone waves. After $1\frac{3}{4}$ hours the beats were distinctly more feeble, and a new mixture was substituted similar to the first, except that the $CaCl_2 = 0.036\%$. The beats, at first somewhat incoördinated, subsequently improved greatly in amplitude and the strip was beating regularly at 6.10 P. M. At this time the strip was transferred for the night to the slow drum. The record upon this drum showed regular and strong beats superposed upon large tone waves for the first five to six hours. Afterwards the tone waves disappeared gradually and the amplitude of the beats also decreased regularly but slowly. At 9.10 A. M., March 10, the solution was changed to one containing 0.046 per cent $CaCl_2$. The strip continued to beat regularly and at first with increasing amplitude; subsequently the size of the beats became smaller, and at 4.30 P. M., they were quite feeble. At 4.30 P. M., March 10, the solution was changed to one containing 0.05 per cent $CaCl_2$. The beats at first increased in amplitude, but subsequently decreased in amplitude, becoming invisible on the record after

five to six hours. Subsequently a few large beats at a slow rate appeared, lasting for about one half hour. The strip then remained quiet until the next morning, 9.15 A. M., March 11. The solution surrounding the strip was then replaced by terrapin serum; no beats were obtained, but this result was inconclusive, as the serum was found to have clotted. (Terrapin serum often shows this peculiarity of successive clottings.) The total record, therefore, of regular contractions of the strip of vein bathed in mixtures of calcium, potassium, and sodium chloride extended from 3.30 P. M., March 9, to 11 P. M., March 10, an interval of thirty-one to thirty-two hours. Subsequent experience indicated

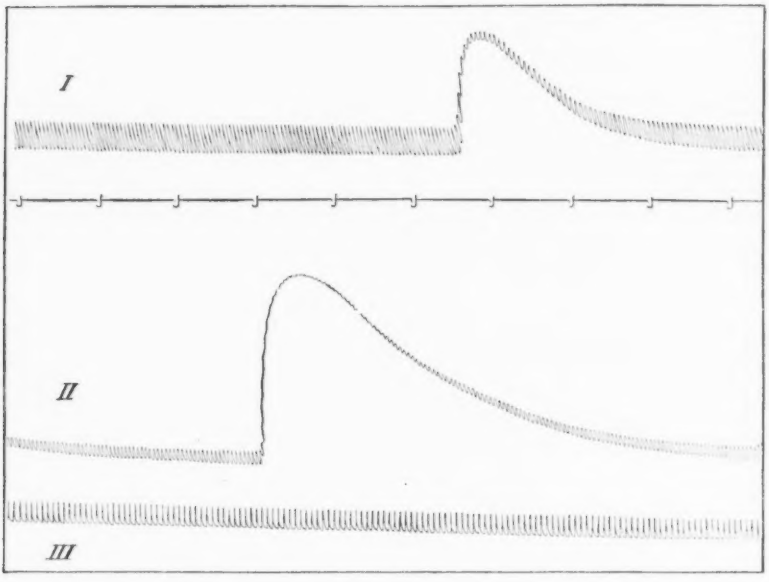

FIGURE 1. Records from experiment of March 9–10, 1898. Seven-ninths the original size. Automatic contractions of a strip of vena cava of the terrapin when immersed in a Ringer's mixture. Curve I, one hour after suspension, $CaCl_2 = 0.026$ per cent. Curve II, three hours after suspension, $CaCl_2 = 0.036$ per cent. Curve III, twenty-three hours after suspension, $CaCl_2 = 0.046$ per cent. The time-record gives minutes, and applies to all the curves.

that a longer record would have been obtained if the percentage of calcium chloride had been still further increased. Records of the contractions were kept during the entire experiment. The rhythm was perfectly regular throughout, but towards the end with slowly changing rate. During the first day, March 9, the rate remained constantly at twenty beats per minute. On the

second day, March 10, the rate steadily decreased, being seventeen beats per minute at 11 A. M., and eight per minute at 4.30 P. M. Specimens of the record obtained at different times are given in the accompanying illustrations.

Experiment March 16 to 19. Strip of vein from right superior vena cava of terrapin. Rinsed in Ringer's mixture and suspended at 11.30 A.M. March 16, in a mixture of NaCl 0.7%, $CaCl_2$ 0.020%, KCl 0.04%; load 100 mgms.

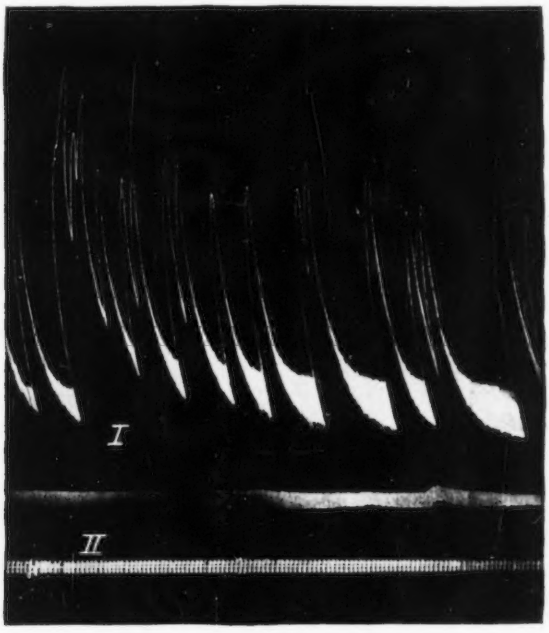

FIGURE 2. Experiment of March 9-10, 1898. Automatic contractions of a strip of vena cava from the terrapin when immersed in a Ringer's mixture. Record on the slow kymographion, giving one revolution in 12 hours. Curve I, 2 to 3 hours after suspension, showing the tone waves. Curve II, 17 to 18 hours after suspension, absence of tone waves. (In part of the record the writing point missed the drum.) The time-record below gives minutes.

Strip began beating at once, showing also large tone waves, rate of beat 14 per minute. At 12.25 A.M. beats feeble; weight increased to 500 mgms., causing an increased amplitude of beat. At 1.20 P.M. rate equal 17 per minute; amplitude of beat 4 mms. At 4.20 P.M. March 16, mixture changed to one containing 0.036% $CaCl_2$, causing increase of amplitude from 1½ mms. to 3½ mms. At 4.40 P.M. load changed from 500 to 100 mgms.; as a result the amplitude of

the beat increased to 6 mms., but afterwards gradually decreased to 4 mms. At 6 A.M. March 16 the strip was changed to the slow drum for the night, and the solution was changed to one containing 0.046% $CaCl_2$. The strip beat throughout the night but exhibited curious periods, groups of scarcely visible beats alternating with groups of much larger ones, the beats in the latter groups increasing gradually to a maximum and then slowly decreasing, after the type of the Cheyne-Stokes respiratory rhythm. At 7.30 A.M. March 17, the strip was beating better than during the night, the solution was changed to one containing 0.056% $CaCl_2$. An increase in the amplitude of the beat followed, which soon, however, passed off, the beats again showing periodic groups of larger and smaller amplitude. At 11 A.M. March 17, the solution was changed to one containing 0.066% $CaCl_2$. A remarkable effect followed. The beats increased at once from a scarcely visible size to an amplitude of $3\frac{1}{2}$ mms., showing also an increased rate (not measurable on the slow drum). The strip in this mixture beat well with force and regularity during the afternoon. At 3 P.M. March 17, the strip was transferred to the faster drum, showing a rate of 9 beats per minute. At 4.40 P.M. again transferred to the slow drum for the night. The beats decreased gradually in amplitude during the night, but were still present at 7.15 A.M. March 18. At this time the mixture was changed to one containing 0.076% $CaCl_2$. This solution caused an increase in amplitude, but at 9.30 A.M. March 18, the beats were becoming irregular although of good amplitude. At 10.45 A.M. the solution was changed to one containing 0.086% $CaCl_2$, but with no beneficial effect after an interval of 20 minutes. The solution was then changed to the original mixture containing 0.026% $CaCl_2$. This solution caused a series of irregular beats lasting an hour. The solution was then diluted with twice its volume of NaCl 0.7%, but without effect. At 1.25 P.M. March 18, the bath was changed to one consisting of clear sheep's serum diluted with twice its volume of NaCl 0.7%. The serum caused at once an increase in tone and a return of contractions; finally, regular strong beats at a rate of 16 to 17 per minute and a maximum amplitude of $3\frac{1}{2}$ mms. These beats continued during the night of March 18 with gradually decreasing amplitude until about 9 P.M. After a long period of rest there was a series of small and infrequent contractions between 5 and 7.30 A.M., March 19. These beats stopped suddenly and subsequent exposure to various solutions failed to produce further contractions.

In this experiment it will be noted that the strip of vein beat constantly although with varying amplitude and rate in a bath of inorganic salts from 11.30 A.M., March 16, to 11.45 A.M., March 18, an interval of forty-eight hours. An interesting result observed in this and in other experiments was that as the percentage of calcium chloride was increased, a certain mixture was obtained (in this exper-

iment NaCl 0.7%, $CaCl_2$ 0.066%, KCl 0.04%) in which the character of the beats was suddenly improved for a long period of time, the beats forming a marked contrast to those obtained with solutions having less or more of the $CaCl_2$. Experience has shown that it does not follow that this beneficial result would have been obtained if the same solution had been used at the beginning of the experiment, but it would seem that after a certain period of activity an increase in the percentage of calcium chloride may at a given concentration call forth renewed contractions of remarkable vigor and regularity. This fact serves to emphasize a precaution which has heretofore been overlooked. In testing the effect of a mixture of these salts in sustaining the contractions of heart muscle it is not sufficient to test simply one mixture and when the heart has ceased to beat in this to conclude that it is exhausted so far as any further action of inorganic salts is concerned. In White's[11] experiments, for example, in exhausting the ventricle upon a Ringer's mixture the following proportion of salts was chosen: NaCl 0.6%, 100 c.c.; $CaCl_2$ 1%, 1 c.c.; KCl 1%, 0.75 c.c.; and $NaHCO_3$ 1%, 1 c.c. When the ventricle had ceased to beat upon this mixture, and in the most favorable cases this happened after nine hours, White drew the unjustifiable conclusion that the supply of serum-albumin in the ventricle was exhausted, and that no further beats could be obtained unless the ventricle was fed with a solution containing serum-albumin. If the author had continued to modify the proportions of salts in his Ringer's mixture he would have discovered doubtless that the ventricle had not been entirely exhausted, or, in terms of the theory he adopts, that some serum-albumin was still present in the heart.

In this experiment it will be noted also that after beating forty-eight hours in a bath of inorganic salts the strip of vein gave good and regular beats for a period of eight hours or more in a bath of diluted blood-serum. A similar result was obtained in other experiments after the strip had contracted for twenty to thirty hours in solutions of Ringer's mixture of salts, and when, apparently, alterations in the proportions of the salts were no longer effective in producing further contractions. Thus in an experiment of March 21–22 the strip was suspended as described above, first, in a mixture containing NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.03%, and this solution was changed at intervals to others with increasing percentages of $CaCl_2$ until the concentration of the $CaCl_2$ was equal to 0.066%. In

this case contractions were obtained from 4.45 P.M., March 21, to 3.15 P.M., March 22, an interval of twenty-two hours and a half. At the end of this period the strip was still beating, although irregularly. The strip was then placed in a bath of sheep's serum diluted twice with 0.7 per cent solution of sodium chloride. The result was an improvement in the beats, which was not, however, very marked and lasted only for three hours. In another experiment lasting from April 12 to April 15, the strip of vein was placed first in a mixture of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.04%; and this was replaced subsequently by other mixtures until the percentage of calcium had reached 0.086. The experiment was begun at 6 P.M. April 12. The tone waves for three to four hours were large, but the beats were small and rapid, and about 5.25 P.M. the next day fell into irregular groups after beating regularly for twenty-three and a half hours. The strip was washed for a few minutes in 0.7 per cent solution of sodium chloride and then transferred to a bath of sheep's serum diluted twice with 0.7 per cent sodium chloride solution. The strip soon recovered, giving regular strong beats that increased slowly to a maximum and then gradually grew smaller, becoming too small to record in about ten hours. By changing the serum bath frequently the strip was kept beating feebly for seventeen hours longer, making a total record of about twenty-seven hours, although for some portion of this time during the night the beats were absent or too small to record. Each renewal of the serum during this interval was followed by a temporary increase in the amplitude of the beats.

This favorable action of blood serum, after apparently complete exhaustion upon Ringer's mixture, has been obtained by Greene upon strips of ventricular muscle, and by Mr. Walden, in experiments not yet published, upon the entire heart of the frog. It may well be that in these cases we have an example of a genuine nutritive action of serum. It is possible that the nutrient substances in the serum, whatever these substances may be, are assimilated by the heart tissue and are constructed into new contractile material. The comparatively brief period, however, that the strips continued to beat well in the serum bath is somewhat opposed to this idea. One would suppose that if genuine nutrition had taken place the recovery of contractility would have been of a more permanent character. Another fact that points in the same direction is that after this exposure to serum subsequent treatment with Ringer's mixture of salts failed to provoke contractions. One would suppose that if nutrition had

taken place the strip would have reacted to this mixture after the manner of a strip freshly taken from the heart. While, therefore, one cannot deny the possibility of a nutritive action of the serum under the conditions described, it remains possible that this action of the serum may be merely stimulating rather than nutritive. It is possible, indeed it is probable, that in blood serum the inorganic salts, whose importance we are insisting upon, exist in a more favorable condition, either as regards their relative proportions, or the form of their combination, than in the simple Ringer's mixture. This explanation of the temporary recuperative action of serum after previous exhaustion upon Ringer's mixtures is one that may be tested more or less satisfactorily by experiments. At present only a single experiment of this character has been completed, but the results of it may be given here entire, partly because it is indicative of the correctness of the hypothesis suggested, and partly because it falls in line with the other experiments just quoted, in showing the great improbability of Kronecker's view that the contractions of heart muscle are supported only by the serum-albumin present in its substance.

In this experiment the strip of vein was first made to beat in a Ringer's mixture of sodium, potassium, and calcium chlorides until no further contractions could be obtained, the total period being a little more than thirty-three hours. The strip was then transferred to a mixture of similar salts containing also some casein that had been purified by several precipitations. The idea underlying this last modification of the Ringer's mixture was that by bringing the calcium into combination with an organic compound it might be more effective in stimulating heart contractions than when in simple inorganic form.

The casein used in these experiments was prepared from milk by the method first described by Hammarsten, as follows: The milk was diluted with four volumes of water and was then precipitated by careful addition of dilute acetic acid. The precipitate was washed freely, by decantation, in a large bulk of distilled water, several litres of water being used. After draining off the water the precipitate was dissolved in water containing a trace of ammonium hydrate, filtered, and reprecipitated by dilute acetic acid. The second precipitate was again washed thoroughly in several litres of water, and the larger portion of it was then dissolved in a measured amount of one quarter per cent solution of sodium carbonate. The solution thus obtained

was used upon the strip of vein, as described below, after being added to a Ringer's mixture of NaCl, $CaCl_2$, and KCl in the proportion of 5 parts of the casein solution to 95 parts of the Ringer's mixture. A portion of the second precipitate of casein was again dissolved in water with a trace of ammonia, filtered, reprecipitated by acetic acid, washed and dissolved in one quarter per cent solution of sodium carbonate. This solution of casein after three precipitations was more dilute than the one used after two precipitations.

As will be seen from the details given below, the strip of vein that had ceased to beat in a series of Ringer's mixtures, again gave regular and strong contractions for a period of nineteen hours in a Ringer's mixture to which the casein solutions had been added, a result as favorable as had been obtained under similar conditions with blood serum. It is perhaps scarcely necessary to add that the good result obtained in this experiment was not due to the sodium carbonate, as was shown by a number of control experiments with Ringer's mixtures containing sodium carbonate. It will also be noted in the experiment given below that after the casein-Ringer mixture had ceased to act upon the strip of vein, diluted sheep's serum was not able to restore automatic contractility, although the strip was still in an irritable condition.

Experiment, April 30 to May 3. A strip of vein was taken from the right superior cava of the terrapin, attached to the lever at 12.55 P.M. April 30, and immersed in a Ringer's mixture of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.04%. The strip began to beat at once, the beats increasing in amplitude to a maximum that was reached in about one-half hour. At 1.30 P.M. the amplitude of the beats was 9 mms. and the rate was 20 to 21 per minute. At 1.45 P.M. the strip began contracting with alternately large and small beats, and tone waves of the usual type appeared. At 3 to 3.30 P.M. April 30 the contractions were again of uniform size, showing an amplitude of 5 mms. and a rate of 18 per minute. At 3.35 P.M. April 30, the record was transferred to the slow drum, and although the contractions were still good and regular a new Ringer's mixture was added, containing 0.036% $CaCl_2$. The contractions continued throughout the night, the amplitude first increasing and then slowly decreasing during eight hours. After this period the contractions were feeble, and fell into groups. At 7.13 A.M. May 1, a new Ringer's mixture was added, containing 0.056% $CaCl_2$. The strip began beating regularly at once, and the record was transferred to the faster drum from 7.20 A.M. to 11.55 A.M. May 1. At 9.50 A.M. and 11.50 A.M. the solution bathing the strip was changed, each time to one containing 0.066% $CaCl_2$. The beats during this period were regular but small, having an amplitude of 2 to 2½ mms. At 11.55 A.M. May 1

the record was again taken upon the slow drum, and at 2.30 P.M. the Ringer's mixture was changed to one containing 0.086% $CaCl_2$. The beats, as the result of this last change, increased in size at first and then became gradually smaller; but regular contractions continued until about 10 to 10.30 P.M. May 1. From this time until seen again at 7 A.M. May 2 the strip showed no beats, but maintained a constant tone. The total time of contracting in Ringer's mixtures with increasing percentages of $CaCl_2$ was therefore from 12.55 P.M. April 30 to 10.30 P.M. May 1, an interval of 33½ hours. At 7 A.M. May 2 the strip was changed from a Ringer's mixture containing 0.086% $CaCl_2$ to a casein-Ringer mixture, containing casein that had been precipitated three times and finally dissolved in one quarter per cent solution of Na_2CO_3. This casein solution had been added to a Ringer's mixture of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.14% in the proportion of 5 parts of the former to 95 parts of the latter. The effect of this solution was to cause a very large increase in tone and regular though small beats. At 9.20 A.M. May 2 the record was changed to the faster drum, a slow increase in tone still continuing. At 9.35 A.M. May 2 the solution bathing the strip was changed to a similar one containing a larger amount, 0.06%, of KCl with the result that while the size of the beat decreased the slow increase in tone continued. At 9.45 A.M. May 2 the strip was changed to a stronger solution of casein (2d precipitation added to a Ringer's mixture containing a larger percentage of $CaCl_2$ — NaCl 0.7%, $CaCl_2$ 0.056%, KCl 0.04%). The beats began to improve at once. At 10.45 A.M. May 2 the bath was changed to a similar solution containing 0.086% $CaCl_2$. The beats at this time had an amplitude of 3+ mms., and a rate of eighteen per minute. The maximum amplitude of beat was reached at 12.45 P.M. May 2, and afterwards the size of the beat gradually decreased. At 5.15 P.M. May 2 the beats were feeble and very slow, two per minute. A new casein-Ringer solution was added at 5.35 P.M. containing 0.126% $CaCl_2$. The beats again increased in amplitude and rate. At 5.50 P.M. May 2 the record was transferred to the slow drum. The beats increased in size to a maximum of 3½ mms. At 9.15 P.M. while the strip was beating well the solution was drawn off, shaken, and again returned to the tube, the change causing a slight temporary increase in the size of the beat. The strip continued to beat but with gradually diminishing amplitude until 2 A.M. May 3. From 2 A.M. to 7 A.M. May 3 the strip gave no contractions. At 7 A.M. changed the casein-Ringer solution to one containing 0.146% $CaCl_2$ and subsequently 0.166% $CaCl_2$, but the strip gave no further contractions. The total period of contractions in the casein-Ringer solution, therefore, was from 7 A.M. May 2 to about 2 A.M. May 3, an interval of 19 hours. After the negative result of the last mentioned casein solution the strip was bathed for a few minutes in 0.7% NaCl solution and subsequently in sheep's serum diluted with twice its volume of 0.7% solution of NaCl. In this solution it was allowed to remain 3¼ hours,

but gave no contractions at all though showing a slight increase in tone. At 10.45 A.M. May 3 tested the irritability of the strip by mechanical stimulation, stretching it slightly by pressing on the lever; the strip responded at once with a good contraction. The serum was now drawn off and the strip at once began to beat regularly, at a rate of 15 per minute, while suspended in air, at the same time showing gradually a marked increase in tone. The beats became smaller as the tone increased, and finally ceased. At 1 P.M. May 3 the strip was again immersed in the diluted sheep's serum. The result was a marked loss of tone but no contractions whatever during a period of immersion of two hours. At 3 P.M. the serum was again drawn off, leaving the strip suspended in air. Again a series of rapid beats, although this time quite small in size, and a marked increase in tone. The beats in this case soon ceased; the increase in tone, however, continued for an hour and then slowly passed off, the strip returning to its original length in about 10 hours. No beats at all occurred after the few initial ones when the strip was first exposed to air. This strip was weighed while moist at the end of the experiment; its weight was equal to 0.014 grms. Some specimens of the records obtained in this experiment are given in the accompanying illustration (Fig. 3).

It is difficult to apply Kronecker's test of what constitutes a genuinely nutrient liquid to the experiments detailed above. His test as originally stated was that the liquid should be able to call forth and sustain contractions of the heart muscle (ventricle) after the latter had been brought to a complete standstill by successive treatments with normal saline followed by normal saline made alkaline with sodium carbonate. It was stated with great positiveness that any liquid capable of producing rhythmic contractions after this treatment must contain serum-albumin. When, however, White recognized that a ventricle that had been brought to a standstill after treatment by the method of Martius was capable of contracting rhythmically for as much as nine hours longer when supplied with a Ringer's mixture, it became necessary to amend the original statement. Following closely the conditions of his experiment, White was forced to assume that the serum-albumin contained in the capillary spaces of the ventricular muscle was more difficult to remove than had been supposed, and that to remove it and bring the ventricle into a state of complete exhaustion or inability to contract, it was necessary to employ successively the treatment with normal saline, Martius's alkaline saline, and Ringer's mixture in the proportion used by White. After such treatment it was stated with the same positiveness as formerly that no liquid could call forth contrac-

tions except one containing serum-albumin. But that this specific conclusion, like the former one, is premature, to say the least, is conclusively shown by my experiments upon the strip of vein, by Greene's experiments on strips of ventricular muscle, and by experiments made in this laboratory by Mr. Walden on the whole heart of the frog. For after exhaustion of the heart muscle by the method em-

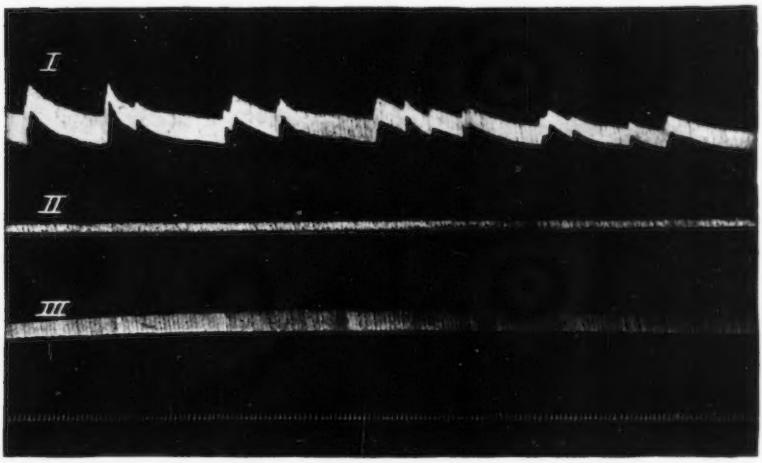

FIGURE 3. Experiment April 30 to May 3, 1898. Seven-eighths the original size. Automatic contractions of a strip of vena cava of the terrapin when immersed in a Ringer's mixture. Records on the slow kymographion giving one revolution in 11 hours. Curve I. Five hours after suspension. $CaCl_2 = 0.036$ per cent. Curve II. Twenty-seven hours after suspension. $CaCl_2 = 0.086$ per cent. Curve III. Fifty-one hours after suspension. The strip at this time was in a bath of Ringer's mixture containing casein (2d precipitate). $CaCl_2 = 0.126$ per cent. The time record on the bottom line gives minutes, and applies to all the curves.

ployed by White, the simple expedient of increasing gradually the percentage of calcium chloride in the Ringer's mixture may call forth rhythmic contractions for many hours, — the length of time varying with different hearts and depending presumably on the previous condition of nourishment.

Whatever may be the explanation of the action of these liquids, the fact that a simple strip of vein continued to beat continuously for forty-eight to fifty-two hours, in liquids that were frequently changed and contained no serum-albumin or related proteid, serves to render the hypothesis of Kronecker in the form stated highly im-

probable. It is scarcely conceivable that the strip of vein soaked for hours in relatively large quantities of liquid should still contain sufficient blood in its substance to supply the energy for such long-continued series of contractions. If one objects that the necessary serum-albumin, although not contained in the blood-spaces of the muscular tissue, may still have been present in the liquids of the tissue, that is, in the tissue lymph, then we are met by a new hypothesis. Apparently it would not be possible to remove completely the serum-albumin from the tissue lymph both extra-cellular and intra-cellular, but if those who believe that serum-albumin furnishes immediately the source of the heart's energy, fall back upon this unprovable hypothesis, one may be justified in insisting on some direct evidence that serum-albumin is directly necessary to, or in any way connected with the contraction of the heart muscle. No positive evidence whatever of this character has yet been submitted. On the other hand, when one considers the wonderful efficiency of Ringer's mixture of inorganic salts in maintaining contractility in the heart muscle, it seems impossible to avoid the conclusion that the energy for these contractions is not furnished by any substance contained in the blood-spaces of the heart, but by material contained within the heart tissue itself. Whether this material is a part of the organized protoplasm of the muscle, or is contained in the liquids of its sarcoplasm or the interstitial lymph, it is not worth the while at present to speculate. It is contained within the heart substance in amounts varying in the hearts of different animals, and, as long as it is present, so-called automatic contractions may be produced if only the proper stimulus is supplied. This position, I take it, is similar to that adopted by Gaule, but disputed by Kronecker, namely, that the heart beats at the expense of its own substance.

As to the nature of the normal stimulus, White [11] admits that the presence of calcium is a necessary condition for contraction. The various experiments described or referred to in this paper upon the effect of artificial mixtures in sustaining the contractions of heart muscle agree in proving the same fact, namely, the necessity of the presence of calcium compounds, and it seems to the author that they indicate, if they do not prove, that the calcium compounds enter into a reaction of some kind with the material furnishing the energy of the contraction, and thereby produce the inner stimulus. As Ringer [7] first showed, the calcium compounds alone, in solutions of sodium chloride isotonic to the blood, are not capable of maintaining a

constant rhythm for a great length of time. The ventricle or strip under such conditions shows a tendency to hurried contractions that pass into a state of strong tone. It would appear, therefore, that while the calcium is probably concerned in some way with the reaction that leads to contraction, yet for the production of the rhythmic sequence of full contraction and full relaxation, a combination of potassium and calcium salts in isotonic solutions of sodium chloride is requisite. We have no facts at present, so far as the author is aware, that enable us to explain, even by a provisional hypothesis, the nature of this supposed interaction of the calcium and the potassium compounds.

The Relation of the Normal Sequence of the Heart-Beat to the Composition of the Circulating Liquid.

In the vertebrate heart each beat begins normally in the great veins leading to the heart and thence spreads to auricle and ventricle. The pulsations of the venæ cavæ or pulmonary veins have been described by a number of observers, and in recent years have been studied with care by Tigerstedt and Stromberg[14] and by Engelmann.[5] According to the latter, the three venæ cavæ and the sinus in the amphibian or reptilian heart beat simultaneously or may so beat, the initial contraction of a heart systole beginning spontaneously in any portion of the venous end of the heart. The subsequent contractions of auricle and ventricle, on the contrary, are not spontaneous, but are initiated by an impulse that starts in the great veins and spreads thence to the auricles and ventricles. The fact that the heart systole always under normal conditions begins at the venous end of the heart and spreads in an orderly manner to the arterial end Gaskell[15] has endeavored to explain by showing that the muscle at the venous end possesses greater rhythmic power. He suggests that in the veins and sinus the structure, arrangement, and properties of the muscle cells are similar to what is found in the primitive contractile tube. The muscle cells of this part of the heart therefore retain the primitive rhythmicity of the heart of the young embryo to a greater degree than the more specialized tissue of the auricle and ventricle.

Influenced by the idea advocated in this paper that the inorganic salts of the blood and lymph of the heart play an essential part in initiating and controlling the heart contractions, the suggestion naturally arose to test the reaction of different parts of the heart toward

Ringer's mixtures of varying compositions, toward serum with varying dilutions, and other liquids. It seemed possible that in this way the greater rhythmicity of the venous end of the heart might be brought into causal relations with the normal composition of the circulating blood, and our understanding of the invariable sequence of the heart-beat advanced a step nearer to its ultimate explanation. Upon consulting the literature in connection with the experimental work undertaken for the purpose described, I have been much impressed with several facts discovered by previous workers that have been more or less overlooked, but which seem to me quite significant in their bearing upon the point under discussion. These facts tend to show that the ventricle when fed with undiluted blood or serum is not capable of giving a series of automatic contractions, but if the blood or serum is diluted with normal saline or replaced entirely by saline or other so-called indifferent liquids the ventricle may be made to give long-continued contractions.

Merunowicz[15] (1875) noticed that the apex of the frog's heart, which normally gives no contractions when fed with undiluted serum, may be made to beat if the serum is diluted four times with physiological salt solution. An experiment is described by Merunowicz that illustrates this difference in behavior of the ventricle, and is further interesting in that it is substantially the same experiment as that performed later by Aubert. In this experiment the heart was kept beating for an hour upon the above mixture of serum and physiological salt solution. The apex was then tied off, with the result that it continued to beat without interruption, although in the normal animal with the heart filled with its own blood such an operation is followed by a stoppage of the apex beats.

This difference between the action of undiluted serum and serum diluted with physiological salt solution upon the contractions of the apex of the frog's ventricle is more clearly brought out by the well-known experiments of Aubert.[16] Aubert made use of the clamping device employed by Bernstein. The apex of the ventricle was clamped off for a number (60) of seconds, with the usual result that when the clamp was removed the apex remained motionless although the rhythm of sinus, auricle, and basal portion of the ventricle was undisturbed. If now a little physiological salt solution was introduced into the ventricle through a cannula placed in the inferior vena cava, the apex began to beat in a few minutes with a rhythm independent of that of the rest of the heart. Then while all parts of the heart

were beating upon the saline mixture, if undiluted serum was again introduced into the heart the apex soon became quiet, with the exception perhaps of an occasional strong contraction while the remainder of the heart continued its rhythmic pulsations. This alternation of serum and physiological salt solution with the above described effects upon the apex could be repeated a number of times.

The present author has had his attention called very often in experiments made during the last four years to this peculiarity in the reaction of the ventricular muscle. Strips of the apex of the terrapin's ventricle suspended by Gaskell's method in a moist chamber will rarely give spontaneous beats, although soaked with their own blood. With the species of terrapin that I have used spontaneous beats indeed were never obtained, although the strips were stimulated rhythmically for hours in the way recommended by Gaskell for strips from the heart of the tortoise. If, however, such strips are suspended in a bath of physiological salt solution, a series of spontaneous beats will always be obtained unless the nutritive condition of the heart is very poor indeed. The beats produced in this way begin a variable time after the immersion in the salt solution, and exhibit a definite curve, the peculiarities of which are described in detail by Greene in an accompanying paper. In the same paper the author proves that strips of ventricle suspended in the animal's own serum do not usually beat. An occasional strong contraction may occur, but not a rhythmic series. The strip may remain perfectly quiet for hours although quite irritable, and at any time it may be made to give a series of beats by diluting the serum sufficiently with physiological salt solution. It will be noted that this latter result is really the same as that obtained by Aubert[16] in clamping off the apex of the frog's ventricle and changing the serum in the heart cavity to physiological salt solution.

Such results lead us directly to the conclusion that the ventricular muscle of the frog's or terrapin's heart does not contract spontaneously when soaked in its own blood or serum, or to express it in another way, the ventricular muscle under such conditions is not capable of automatic activity. The whole heart, on the contrary, beats when filled with its own blood or serum, or when supplied artificially with the blood or serum of other animals. It follows, therefore, that other parts of the heart toward the venous side are capable of automatic activity when bathed in blood or serum. By means of

experiments made on strips from the large veins opening into the heart, I have been able to show this difference in reaction between the tissues at the two ends of the heart in a very striking way.

In these experiments strips of the right superior vena cava, of the apex of the ventricle, and usually of the auricle also, were taken from the same animal and attached in the way previously described to recording levers. Usually the strip of vein was placed under a tension of 100 to 500 mgms., that from the auricle under a tension of one gram while that of the ventricle was loaded with two grams. The strips were immersed in a bath of serum from the blood of the same animal, or in horse's or sheep's serum, or finally in baths of Ringer's mixture of inorganic salts in varying proportions. Careful records of the contractions obtained were taken upon the kymographion during a period of from one to three days. In all, seventeen experiments of this character were completed, but as the records are voluminous on account of the long periods of time required, it is perhaps sufficient to state briefly the character of the results obtained and illustrate these results with specimens of some of the curves.

When immersed in undiluted serum either of the terrapin's or horse's blood, a strip of muscle from the apex of the terrapin's heart does not usually give rhythmic beats. A few isolated strong contractions may occur, but these are rare and take place at long intervals, and in my experiments usually happened immediately or shortly after immersion. As a rule the strip remains perfectly quiet for hours, although very irritable toward mechanical stimulation. From this condition of rest it may be aroused into rhythmic activity by substituting physiological salt solution for the serum, by diluting the serum sufficiently with the physiological saline, or by adding a certain excess of calcium chloride to the serum. Diluting the serum with 0.7 per cent solution of sodium chloride was usually more effective in producing a long series of contractions than the addition of calcium chloride. The amount of dilution required to start the strip into a sustained series of contractions varied in different cases. Sometimes dilution with an equal volume of the 0.7 per cent solution of sodium chloride sufficed, but in other cases dilution with twice the volume or more was necessary. The same variation was observed in the amount of calcium chloride which it was necessary to add to produce contractions; addition of as much as 4 c.c. of a one per cent solution of calcium chloride to 100 c.c. of serum was in

some cases entirely ineffective, while 6 or 8 c.c. would call out a series of beats.

A strip of the right superior cava near the sinus on the other hand usually gave rhythmic beats for a variable period, when immersed in undiluted serum. In some cases visible contractions disappeared in two hours or less, in other cases the strip maintained beautifully regular and strong contractions for as long as 24 to 30 hours. A specimen of one of these records is reproduced in Fig. 4. In this case the strip of vein was bathed in serum from its own blood. The illustration shows not only the regular and sustained rhythm of

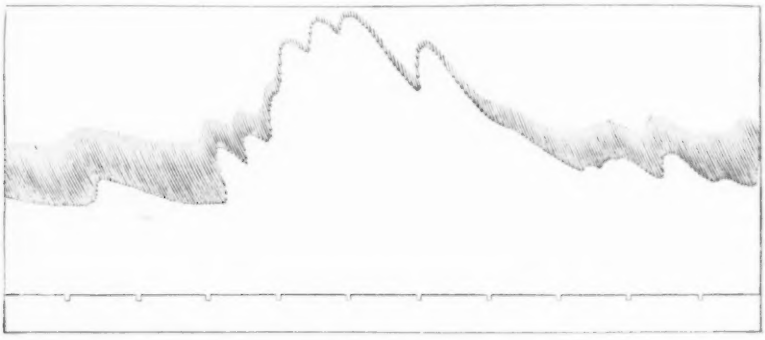

FIGURE 4. Automatic contractions of a strip of vena cava of a terrapin when immersed in a bath of its own serum. Seven tenths original size. Experiment of March 6, 1898. The time record indicates minutes.

the beats, but also the marked variations in tone, which were practically a constant phenomenon in the records taken with the strips of vein. Whether the strip was immersed in serum or in a Ringer's mixture these marked variations in tone were observed. In some cases they were very pronounced and developed at long and irregular intervals. In other cases they were less extensive or occurred at short intervals and with almost perfect regularity. If the strip was beating, the contractions were superposed on the tone waves, growing smaller as the tone of the strip increased and increasing in amplitude as the strip relaxed. When beats were absent the tone waves were still present, as a rule, and indeed in some cases exhibited an extraordinary range (see Fig. 5).

In the longer experiments, especially in those in which the bath was composed of Ringer's mixture of salts, it was observed that the

wave-like variations in tone increased in amplitude for some hours and then slowly passed off, becoming both smaller and less frequent, so that after twelve or more hours the strip might be beating with as little variation in tone as in the case of the ventricular strip. In this last condition the vein strip was still liable to marked changes in tone if the conditions to which it was exposed were altered. A careful study was not made of the nature of these conditions, but it was

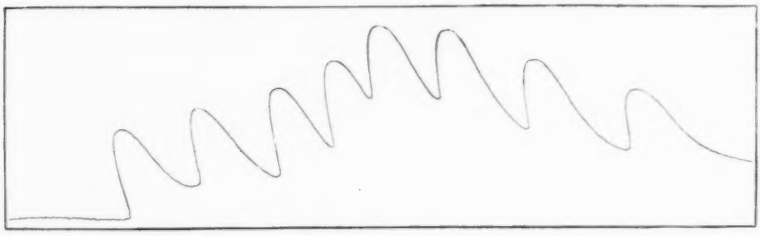

FIGURE 5. Specimen of rhythmic tone waves superposed on a larger tone wave. Seven elevenths the original size. Strip of vena cava of terrapin in a bath of Ringer's mixture consisting of NaCl 0.7%, KCl 0.03%, $CaCl_2$ 0.026%. The strip was giving very small beats just visible in places on the record. Experiment, Feb. 7 to 9, 1898.

observed among other things that aërating the liquid or exposing the strip to air caused usually a strong increase of tone. The susceptibility of the muscular tissue at the venous end of the heart to changes in tone is evidently one of its most striking properties, and one that merits especial study, since it is possible that under normal conditions it may exercise an important regulating influence on the blood flow through the heart. This point, however, was not studied in this investigation, and is mentioned here only incidentally because of the constant appearance of tone waves in all the records.

The facts given above with regard to the behavior of strips of the vena cava and the ventricle in undiluted serum taken in connection with the results described by Aubert and others, justify us in concluding that the normal composition of blood is such as to prevent automatic activity on the part of the ventricle, at least in the frog and terrapin, while, on the contrary, it induces automatic activity in the tissue at the venous end of the heart. Whether the action in the latter case is exerted immediately upon the muscular tissue or indirectly through the intrinsic ganglia is a secondary matter from our present standpoint.

Strips of auricle were used in these experiments under the same conditions as those to which the strips of vein and of ventricle were exposed. The results, however, were not so uniform, and in the earlier experiments were complicated by the fact that proper precautions were not observed to ensure that none of the sinus musculature was included in the strip taken. It may be said, however, that in general the reactions of the strips of auricle were somewhat intermediate in character between those exhibited by the strips of vein and ventricle.

In some cases the auricular strip gave rhythmic beats in undiluted serum, but these were slower although much stronger than those given by the vein strip. In other cases the auricular strip remained quiet in the undiluted serum, except for changes of tone, but gave a long series of beats when the serum was diluted with physiological salt solution. Dilution with this latter solution, moreover, seemed to cause rhythmic contractions in the auricular strip more easily, that is, with a smaller degree of dilution than in the case of the ventricular strip. In the matter of tone changes also the auricular strip exhibited intermediate properties. Its tone changes were marked, and indeed practically a constant occurrence in the records taken, but they were less pronounced than in the case of the strips of vein.

In addition to the experiments with diluted and undiluted serum, numerous experiments were made in which the bath was composed only of Ringer's mixture of salts in varying proportions. The results of these experiments showed clearly that it is possible to select such a proportion of calcium chloride and potassium chloride in an isotonic or nearly isotonic solution of sodium chloride as will keep the strip of vein beating for hours, but will cause no contractions at all in the ventricular strip. In a mixture, for example, consisting of NaCl 0.7%, $CaCl_2$ 0.026%, and KCl 0.03 to 0.04%, the strip of vein practically always gave rhythmic contractions together with tone waves that might last for hours (see Fig. 6). In the single experiment in which a negative result was obtained with a mixture of the above composition, it is probable that the strip was injured in some way during the process of removal and suspension, since in this case the tone changes were also very slight and infrequent.

In this same mixture of salts the strip of ventricle as a rule gave either no contractions, or if it contracted at all gave only a few beats or small groups of beats separated by long intervals of rest. From this resting condition the ventricular strip might be stimulated to a

rhythmic series of beats either by diluting the Ringer's mixture more or less with 0.7 per cent solution of sodium chloride, or by increasing in it the percentage of calcium chloride.

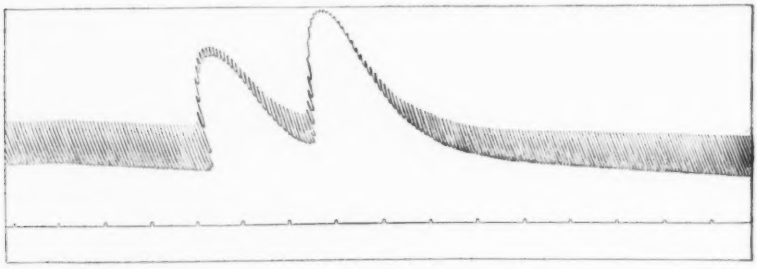

FIGURE 6. Automatic contractions of a strip of vena cava of the terrapin immersed in a bath of Ringer's mixture of the composition NaCl 0.7%, KCl 0.03%, $CaCl_2$ 0.026%. Seven tenths the original size. Experiment of March 21, 1898. The time record indicates minutes.

In addition to the experiments made with strips of ventricular muscle, I have collected a number of observations made during class experiments in which the entire ventricle was employed. In these experiments the ventricle was ligated upon a perfusion cannula, the ligature being laid in the auriculo-ventricular groove. Through the cannula it was irrigated freely with a supply of Ringer's mixture of salts of the composition stated above and under a constant pressure of two to three centimetres. The ventricle was placed in a tonometer filled with 0.7 per cent solution of sodium chloride, and the interior of the tonometer was connected by a wide tube with a test tube suspended by a spiral so arranged that the pressure in the tonometer remained constant in spite of variations in volume of the ventricle. The movements of the test tube were recorded upon a kymographion, and gave the exact changes in volume of the ventricle. The usual result was that when the Ringer's mixture contained 0.026 per cent calcium chloride and 0.03 to 0.04 per cent potassium chloride, the ventricle refused to beat. When, however, the Ringer's mixture was diluted sufficiently with 0.7 per cent solution of sodium chloride, rhythmic, long-continued pulsations ensued. The Ringer's mixture, in other words, behaved precisely like the undiluted serum or blood in the experiments made by Merunowicz and others. The amount of dilution necessary to call forth sustained contractions varied somewhat with the different hearts used.

On the other hand, if the entire heart (sinus, auricles, and ventricle) of a frog or terrapin is isolated and supplied by an artificial circulation of Ringer's mixture of the above composition, the result is that the heart beats quite as well, if not better, than when supplied with serum or blood. We find, therefore, that the reaction of ventricular strips, ventricle, and the entire heart towards a Ringer's mixture of the composition specified is practically identical with the reaction toward undiluted serum. This similarity is most interesting when we remember that the Ringer's mixture chosen contains sodium, potassium, and calcium, in substantially the same proportions as in mammalian blood serum. Whether there is any variation from these proportions in the serum of the terrapin cannot be stated except in the case of the calcium, since complete analyses of the terrapin's serum are lacking. Mr. Greene's determination of the calcium in the serum of the terrapin shows that it exists in the same average percentage as in mammalian serum. For the terrapin's plasma Greene found 0.0131 gms. calcium oxide to each 100 c.c. of plasma, while for mammalian serum Gerlach [19] reports (analyses by Drechsel) for the dog's serum 0.014 gms. calcium oxide, and Abderhalden [20] in some recent analyses gives for ox serum 0.01194 per cent of calcium oxide, and for horse's serum 0.01113%.

It may be assumed that the Ringer's mixture in question contains the bases, sodium, potassium, and calcium in approximately the same percentages as the animal's own blood, and certainly there can be no question that it makes, so far as the entire heart is concerned, a circulating liquid of wonderful efficiency in maintaining rhythmic contractility. The author has in fact been accustomed for some years to use a mixture of practically this composition in class experiments for students in place of dilute serum, and it has always worked very satisfactorily even when the experiments, as in the determination of the effect of temperature on the heart rate, have extended over a number of hours.

From the experiments and results quoted in the first part of this paper the conclusion was drawn that the rhythmic automatic activity of heart muscle is in reality dependent upon the presence of a proper proportion of potassium and calcium compounds in the liquids permeating it, and that of these two compounds the calcium in some way has an intimate connection with the liberation of the stimulus causing contraction. If this conclusion is justified, the facts stated in the second part of the paper serve to make reasonably clear the

cause of the normal sequence of the contractions that sweep over the heart from venous to arterial end. As Gaskell and others have shown, the explanation of this regular sequence is to be found in the greater rhythmic power of the tissue at the venous end of the heart, but the cause of the greater rhythmic power in turn is to be sought in the adjustment of the tissue at that point to the relative proportions of calcium and potassium compounds in the liquids of the blood or lymph. This proportion is such that in the heart of the frog and terrapin the blood is capable of liberating a rhythmic stimulus to the muscular tissue of the veins and sinus, but is ineffective toward the ventricular muscle. With such an adjustment the result must be that under normal conditions a heart-systole begins always at the venous end.

On the other hand the ventricular muscle also is capable of so-called automatic activity if only the liquid bathing it have the proper composition, but in blood or lymph or an artificial solution in which the calcium and potassium constituents have the same proportions as in blood it is not automatic. Under these conditions its contractions depend upon a stimulus conducted to it from the auricles, which in turn are stimulated from the sinus, as Gaskell, Engelmann, Krehl and Romberg,[18] and others have contended.

Whether or not these facts hold in the case of the mammalian heart cannot of course be determined without direct experiments on the subject. The well-known experiments of Wooldridge and of Tigerstedt indicate that the ventricle of the mammalian heart, unlike that of the cold-blooded heart, continues to beat, although with a slower rhythm, after separation from the remainder of the heart. This result has been confirmed also by the later experiments of Krehl and Romberg. In all of these experiments, however, a portion of the auricular musculature was left in connection with the ventricle so that the isolation was not complete. Moreover, it is not clear that in these experiments the ventricle continued to beat sufficiently long to establish beyond doubt its power to give automatic contractions under the normal conditions of the circulation. Apparently a much stronger proof of the automaticity of ventricular muscle under normal conditions of circulation is furnished by the interesting experiments of Porter[21] made upon isolated portions of the mammalian ventricle supplied by an artificial circulation of the animal's own serum or blood. In these experiments, however, if I do not misunderstand the author's description, the blood or serum was usually diluted

more or less with isotonic solutions of sodium chloride.[1] It is possible of course that the difference in automaticity exhibited by the venous and the arterial end respectively of the cold-blooded heart may be less pronounced although still present in the mammalian heart. On general grounds we should suppose that the cause of the sequence of beat would be the same in both cases.

There is one point mentioned frequently in this paper that merits an attempt at an explanation. I refer to the fact that an entire ventricle or ventricular strip which will not beat when immersed in blood or serum or when exposed to air will give a definite series of beats when bathed in blood that has been diluted sufficiently with physiological salt solution, or better when immersed directly in the salt solution. When the salt solution is used alone the striking feature of this series of beats is the definite curve it presents. The beats begin with a certain amplitude somewhat sub-maximal, increase in size for a short time, and then decrease regularly and rather rapidly to zero. The actual number of beats varies greatly for different hearts, but the series shows always the same characteristic curve. In the accompanying paper by Greene the details of this curve are described and figured. The point that I wish to refer to here especially is the cause of this series of beats. If one accepts the theory of the causation of the heart-beat that I have ventured to propose in this paper, a credible hypothesis may be suggested to explain this interesting effect of isotonic solutions of sodium chloride.

The ventricular muscle is normally saturated with lymph and blood in which the calcium and potassium constituents exist in proportions that neutralize the stimulating effect of the calcium compounds. When, however, the ventricular tissue is placed in a solution of sodium chloride that is isotonic to the blood, a diffusion of the calcium and potassium constituents may be supposed to take place from the liquids of the tissue to the surrounding bath of sodium chloride. If, as may also be assumed, the rapidity of diffusion of the calcium constituents is less than that of the potassium constituents the normal balance between them is disturbed and a preponderance of the calcium compounds results sufficient to stimulate the muscle to contraction. As diffusion proceeds, the contractions will grow smaller and eventually will disappear, when the diffusible calcium and potassium compounds in the tissue fall below a certain amount in

[1] Dr. Porter has since informed me that he has fed the apex of the dog's heart with dog's blood or serum and obtained rhythmic contractions.

consequence of the large bulk of outside liquid against which the diffusion takes place. In this condition of sodium chloride exhaustion, as it has been called, a temporary recovery may be obtained, as Gaule and Martius showed, by the action of small amounts of sodium hydrate or sodium carbonate in isotonic solutions of sodium chloride. The explanation of this partial recovery given by Martius,[10] namely, that the alkali removes more effectually the carbon dioxide of the tissues and thus prevents local asphyxia, is perhaps sufficient. But the recuperating effect of the alkaline saline is slight at the best, and the important fact is that from this condition of exhaustion so-called the muscle may be aroused into rhythmic activity lasting for many hours by exposing it to the action of a liquid containing the proper proportions of calcium and potassium salts, such as blood or lymph or the artificial solution devised by Ringer.

Conclusions.

1. A strip of vena cava from the heart of the terrapin may be kept in continuous rhythmic pulsation, during forty-eight hours or more, when immersed in baths containing only the inorganic salts, sodium chloride, potassium chloride, and calcium chloride. Since in such preparations there is no mechanical obstacle to the complete removal of blood from the substance of the strip, the result renders improbable the theory advocated by Kronecker that the cardiac tissue beats only as long as serum-albumin is supplied to it by the blood.

2. The muscular tissue of the heart derives the energy for its contractions from material contained within its own substance, and if supplied with an adequate stimulus will continue to contract until this material is consumed, whether or not the organic constituents of the blood are present.

3. Under normal conditions, the stimulus that leads to a heart contraction is dependent upon the presence of calcium compounds in the liquids of the heart; but for rhythmic contractions and relaxations, a certain proportion of potassium compounds is also necessary.

4. An adequate artificial circulating liquid for the whole heart, or for the tissue at its venous end, is a solution of sodium chloride isotonic with the blood, and containing calcium and potassium as chlorides in the proportions, so far as the bases are concerned, found in normal blood. The sodium chloride seems to be essential only in preserving the osmotic relations between the tissues and the surrounding liquid.

5. The muscular tissue of the ventricle of the frog and terrapin is not able to contract automatically when exposed to undiluted blood or serum, or to a Ringer's mixture containing sodium, potassium, and calcium in the proportions present in blood.

6. The tissue at the venous end of the heart (vena cava) is capable of giving sustained automatic contractions in undiluted blood or serum, or in a Ringer's mixture containing sodium, potassium, and calcium in the proportions found in blood.

7. The normal rhythm of the cold-blooded heart depends upon the fact that in blood and lymph the proportion of calcium and potassium compounds is such as to cause rhythmic activity of the muscular tissue at the venous, but not at the arterial end of the heart.

REFERENCES.

[1] HALLER: Elementa physiologiæ corporis humani, 1757, tom. i, lib. iv, p. 493.

[2] MÜLLER: Elements of physiology, 1843.

[3] BUDGE: Wagner's Handwörterbuch der Physiologie, 1846, iii, 1, p. 443.

[4] LANGENDORFF: Archiv f. Anat. u. Physiol., physiol. Abth. (Suppl. Bd.), 1884, p. 1.

[5] ENGELMANN: Archiv f. d. ges. Physiol., 1897, lxv, p. 109.

[6] HOWELL and COOKE: Journal of physiology, 1893, xiv, p. 198.

[7] RINGER: Journal of physiology, 1883, iv, p. 29, and p. 370; 1884, v, p. 352; 1885, vi, p. 154; 1886, vii, p. 291; 1887, viii, p. 20, and p. 288; 1893, xiv, p. 125; 1894, xvi, p. 1; 1895, xviii, p. 425.

[8] MERUNOWICZ: Arbeiten aus d. physiol. Anstalt zu Leipzig, 1875, p. 132.

[9] VON OTT: Archiv f. Anat. u. Physiol., Physiol. Abth., 1883, p. 1; KRONECKER and POPOFF: *Ibid.*, 1887, p. 345; Zeitschrift fur Biologie, 1888, xxv, p. 427; BRINCK and KRONECKER: *Ibid.*, 1887, p. 347.

[10] MARTIUS: Archiv f. Anat. u. Physiol., physiol. Abth., 1882, p. 543.

[11] WHITE: Journal of physiology, 1896, xix, p. 344.

[12] GAULE: Archiv f. Anat. u. Physiol., physiol. Abth., 1878, p. 291.

[13] STIENON: Archiv f. Anat. u. Physiol., physiol. Abth., 1878, p. 263.

[14] TIGERSTEDT and STRÖMBERG: Bihang till K. Svenska Vet. Akad. Handlingar., Band 13, Afd. IV, Nr. 8, Stockholm, 1888. (Quoted by Engelmann, Archiv. f. d. ges. Physiol., 1897, lxv, p. 109.)

[15] GASKELL: Journal of physiology, 1883, iv, p. 43; and Archives de physiologie, 1888, p. 56.

[16] AUBERT: Archiv f. d. ges. Physiol., 1881, xxiv, p. 357.

[17] KRONECKER and MCGUIRE: Archiv f. Anat. u. Physiol., physiol. Abth., 1878, p. 321.

[18] KREHL and ROMBERG: Archiv f. exp. Path. u. Pharm., 1892, xxx, p. 49.

[19] GERLACH: Arbeiten aus d. physiol. Anstalt zu Leipzig, 1872, p. 99.

[20] ABDERHALDEN: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 521.

[21] PORTER: The journal of experimental medicine, 1897, ii, p. 391; and This journal, 1898, i, p. 511.

ON THE RELATION OF THE INORGANIC SALTS OF BLOOD TO THE AUTOMATIC ACTIVITY OF A STRIP OF VENTRICULAR MUSCLE.

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IT is the intention in this paper to investigate more fully the conditions of automatism in the tissue of the heart rather than to discuss the question whether the automatism of the heart resides primarily in the muscular tissue or in the nervous mechanism. Brown-Séquard[1] pointed out more than fifty years ago that the more fundamental question regarding the heart's activity is not which tissue is automatic but why or under what conditions either tissue shows automatism. I emphasize the idea of conditions of automatism with intention, for while one may justify himself in setting forth hypotheses for the explanation of results obtained under the multiple conditions of experimentation, still one must fully recognize the impossibility of reaching a decisive solution of a question which at the present time involves so many necessarily unknown factors.

The influence of the blood on the heart's contraction must necessarily be considered both from the physical and from the chemical point of view. The characteristics of the blood are isotonicity with the living tissue, a certain degree of viscosity, and a complex chemical composition. Since the experiments of Nasse[2] in 1869, on the effects of different strengths of sodium chloride solution on the irritability of the gastrocnemius of the frog, isotonicity with the living tissue has been recognized by all physiologists as a requisite of any artificial solution adapted to sustain the activity of living tissue. In the experiments recorded in this paper, the effort has been made to keep this factor constant by using solutions as nearly isotonic as possible, except in a very few special cases. Certain investigators, notably Heffter,[14] have insisted upon the importance of a degree of viscosity in an artificial circulating fluid. Others have disproved, or at least have thrown grave doubts on the validity of this conclusion;

and until further evidence is brought forth we may safely ignore this property in any investigation on the chemical constitution of the blood in its relation to tissue activity.

As a basis for the preparation of artificial solutions of the salts found in blood chemical analyses of blood or serum as given in the literature of the subject are in many respects incomplete. The most common defect is the lack of information as to the exact forms of the salts present, and the proportions in which they exist as free salts and as salts in combination with organic components. Then, too, such salts as are found only in minute quantities have not been quantitatively determined, although from a physiological point of view they may be of very great importance.

The most abundant salts of blood are those of sodium, potassium, calcium, and magnesium. In experiments up to the present, the action of these salts has been studied mainly upon the heart of the frog. The experiments described in this paper were confined to the terrapin, and were performed exclusively on strips of muscle cut from the apex of the ventricle. The strips were taken either from ventricles filled with blood or from ventricles freed of blood as far as possible by irrigation with normal saline solution. In the latter case the heart was isolated and irrigated through an inflow cannula inserted into the left vena cava and an outflow cannula in one of the aortic branches. The coronary arteries vary much in their origin in the terrapin used, but care was always taken to establish as good a coronary circulation as possible by cutting one of the coronary veins.

From two to four slender strips were prepared from each heart by first cutting off the apical two thirds and then splitting this into the requisite number of pieces. In all, over fifty different hearts were used, giving from two to four strips each, and each strip was submitted to from five to ten distinct changes in the experimental conditions. The effect of each change was observed for periods varying from one to many hours. The average duration of experimentation on a single strip was from forty to fifty hours, although many experiments exceeded seventy-two hours and some reached ninety-six to ninety-eight hours. It will readily be seen that the total time of suspension and the number and character of changes preceding any particular test are most important factors in determining the results of that test, and that the unifying of the results of the entire series of experiments is correspondingly difficult.

Methods and Apparatus.

In 1880 Gaskell demonstrated that a strip of the apex of the terrapin's ventricle would give automatic rhythmic contractions when suspended in a moist chamber and left to itself. This simple experiment offers a method for the study of rhythmic muscular tissue as such. Although an intricate network of nerve fibres interlaces among the muscle cells of the apex of the ventricle, and, according to Berkley,[18] an occasional isolated nerve cell is found in even the apex in the frog and the mouse, still it can scarcely be supposed that a stimulus that acts to produce rhythmic contractions in the isolated strip of the ventricle acts on the terminal nerve elements to the exclusion of the muscle cells. However this may be, whether the automatism resides in the muscle or nerve, the phenomena associated with the rhythm of an isolated strip of muscle seem to admit of a more definite study than the phenomena associated with the activity of the complexly organized heart itself.

In a preliminary experiment made upon a different species of terrapin from that used in the remainder of the experiments, the apex muscle strip of the terrapin's ventricle suspended in a moist chamber remained alive and gave off rhythmic contractions for three days. This preliminary test fully demonstrated the utility of the method, and I have, therefore, adopted this method for the study of the inorganic constituents of blood in their relation to the activity of cardiac muscle. The following pages will, I hope, fully demonstrate the directness, reliability, and simplicity of the method.

My plan of procedure has been, in brief, to suspend a slender strip of the apex of the ventricle (approximately 2.5 cm. long and weighing 0.2 to 0.3 gram) in a moist chamber, attach it to a recording lever under a definite tension, submit it to an artificial fluid by immersing it in the solution or by moistening it in air, and record the resulting phenomena by the lever and by direct observation. The extraordinarily long life of the muscle under such conditions permits a series of experimental changes affecting each strip. By this method one has the very great advantage of placing several strips from the same heart simultaneously in different solutions. The requirements of this method are very simple, and no intricate apparatus is needed. It is necessary only that the muscle be protected from evaporation, and that it be connected with a delicately poised recording lever. The apparatus I adopted has the

additional advantage of all the desirable mechanical conveniences in adjustment.

The muscle was suspended in a glass tube fourteen centimetres in length and of a diameter of one and a half to three centimetres, according to the experiment. The tube was closed at the bottom by a rubber stopper containing a small glass cannula, so arranged as to permit the filling or the complete emptying of the tube without disturbing the heart strip. One side of the upper or inner end of the cannula was drawn out and bent into a small hook for the attachment of the lower end of the muscle strip. The upper end of the heart tube was either covered with oiled paper, or — in the tube used for air experiments — was constricted into a small neck with funnel-shaped outer end, which when filled with a drop of water offered no resistance to the movement of the thread passing through it, yet at the same time prevented evaporation from the heart tube. Silk ligatures were tied around each end of the muscle strip, and one thread closely looped over the glass hook at the top of the stopper-cannula while the other was carried to a lever above, or small glass S-shaped hooks were caught into the ends of the muscle strip. The hooks are preferable for the comparatively large ventricular strips.

A light straw lever was used in these experiments. The lever was balanced on a steel rod one millimetre in diameter by means of a glass hub about twenty-five millimetres long, the ends of which were constricted so that the weight of the lever rested on a very small bearing surface. This device reduced the friction of the lever on the steel wire support to a minimum. The fulcrum of the lever was placed between the attachment of the muscle and the writing point, converting the downward pull on the lever into an upward stroke on the recording cylinder. The lever carried a five-gram weight near the supporting glass hub so adjusted that the total weight on the muscle was approximately one gram. The axis of the lever was supported above the heart tube by means of rods and corks so arranged that the lever could be readily adjusted in any plane. The horizontal adjustment was found extremely convenient, since by it the recording lever could be taken from the recording surface or returned at any moment without in the least disturbing other pieces of apparatus attached to the same stand.

A slow drum was found best adapted to the slow rate usually given by the terrapin's ventricular strip, especially when the experiment con-

tinued through a series of hours including, perhaps, two or three consecutive nights. The particular drum used for most of these experiments had a circumference of forty-seven centimetres, and made one revolution in eleven hours. When parallel experiments were made, as was always the case except in one or two experiments, two and three heart tubes with levers were attached to the same stand, the levers writing one above the other.

The artificial solutions used in these experiments were in every case made in water distilled in glass. The absolute necessity of this precaution in any series of experiments involving the effects of the inorganic salts of blood on living animal tissue was first demonstrated by Locke,[16] whose work has recently been confirmed by Ringer. These investigators showed that minute traces of certain of the heavy metals, such as copper, dissolved from the copper-receiving tanks so often used for distilled water, may completely obscure the effects of the particular salt experimented with. The water used in my experiments was prepared in part by the method devised by Jones and Mackay,[19] except that potassium bichromate was substituted for permanganate of potassium in the first flask according to a recent suggestion of the authors', and that I used a Jena glass condenser tube instead of the block tin tube used by them. More recently the water used was purified by making ordinary laboratory distilled water slightly alkaline with sodium hydroxide and re-distilling in glass.

The sodium and potassium salts used were purified by repeated recrystallization of "chemically pure" salts. Solutions were made by dissolving a weighed quantity of the dry salt in a measured quantity of distilled water. Stock solutions of one per cent strength were prepared from all salts, except, of course, sodium chloride, and solutions for immediate use were made up from these stocks as needed. In the case of the deliquescent calcium chloride a solution was prepared of approximately the strength required, and the amount of calcium in this solution was quantitatively determined by the usual gravimetric method of precipitation as calcium oxalate and conversion to calcium oxide. From this general solution a one per cent stock was prepared.

In the details of experiments the proportions of the various salts used in any given artificial solution are expressed in terms of percentage.

SERUM.

Before discussing the relation of solutions of the inorganic blood salts to the action of a ventricular strip, let us first consider the behavior of such a strip immersed in its own serum. If an apex strip cut from the heart of a terrapin, *Chrysemys picta*, is suspended in a heart tube and covered with normal terrapin serum, absolutely no rhythmical contractions are developed. If the preparation is not quickly made and is allowed to evaporate slightly during the process, a few single contractions may occur when the strip is first suspended. The number of such contractions does not exceed ten or fifteen, and they occur during the thirty to sixty minutes following suspension.

In five experiments strips were kept in serum from twenty-one to one hundred hours. After thirty to fifty hours an occasional contraction may occur. The longer a strip is kept in serum the more frequent the contractions become, but they do not on an average exceed one an hour in strips kept for a very long time. One strip, number 47*d*, left undisturbed for seventy-six hours in serum, gave during that time eighty-two single contractions, seventy-five of these occurring during the last half of the time. Meanwhile bacteria appeared in the serum; this was therefore drawn off and the strip left suspended in air, though still moist with serum. The muscle at once began to contract with full normal contractions and continued to do so for six hours, when the recording drum stopped and further record was lost. Another strip was kept in serum for ninety-six hours, and gave only forty contractions during about one half of the time, the record being lost at intervals during the experiment. At the end of ninety-six hours this strip was quiet in a relaxed state, but when changed to 0.6 per cent sodium chloride solution it immediately gave a series of rhythmical contractions. These contractions were more irregular in rate than is usual in a sodium chloride series, such as will be described later. They were at first 1.2 to 1.4 cm. high, and gradually decreased to zero within an hour and a half. After one hundred hours a bath of Ringer's solution produced a strong increase in tone and fibrillation, showing that the muscle was still alive and irritable.

From these examples it will be seen that *normal serum preserves the heart strip in good condition for contraction for a very long time, but does not supply the necessary conditions for the development of rhythmic contractions of ventricular muscle.*

It is well known that an isolated apex of the frog's ventricle will not give contractions when the heart is filled with its own blood, a fact made use of since its discovery by Bernstein in 1876 to support the view that the contractions of the heart are nervous in origin. It is significant, therefore, in light of what I shall present later, that the ventricular strip agrees in its reaction to serum with the apex of the frog's heart isolated by Bernstein's method.

Modified serum. — In 1875 Merunowicz [4] found that blood of the sheep or rabbit is most favorable to the development of good contractions in the frog's heart when diluted with 0.6 per cent sodium chloride solution in the ratio of one part of blood to four parts saline. McGuire [5] in 1878 investigated the relative proportions of rabbit serum and 0.6 per cent saline in reference to their sustaining power on the frog's heart. He found that a mixture of serum and saline in the ratio of one to six does not produce maximal contractions, — that one to two is better, while greater concentration of serum is "unfavorable." At the present day, following these and similar results obtained by other observers, it is customary to dilute rabbit or other mammalian blood with sodium chloride solution when it is to be used for artificial circulation experiments, without inquiring why the dilution improves the blood in its adaptability to the production of good rhythmic contractions. I have repeated on the apex strip the experiments of McGuire on the frog's heart, and have other experimental evidence which I hope will show to what dilute serum owes its efficiency. It has already been shown that the terrapin's own serum in its normal concentration and composition as well as mammalian serum is inefficient in developing rhythmic contractions in a heart strip.

Experiment 24, December 11, 1897. A perfectly fresh heart strip was suspended in fresh serum obtained from another terrapin. During the succeeding forty-one hours, twelve contractions were developed. These contractions varied in amplitude from 0.1 cm. to 1.0 cm., and occurred at very irregular intervals. The smaller contractions were submaximal. Sodium chloride 0.6 per cent was then added at successive intervals during ten minutes. After twelve minutes full, strong contractions began which were at first 1.0 cm. high but increased to 1.2 cm. in two or three minutes, then slowly decreased to 0.8 cm. in the two hours following. The rate was quite irregular in this series and the contractions were in groups of varying rates. Ten minutes after the series began one group showed a rate of twenty-eight per minute. As the height decreased the rate became slower and slower. After two

hours contractions came only at long intervals and so continued for twenty-four hours.

Experiment number 30 b. An apex strip was immersed in 0.64 per cent sodium chloride solution until the contractions resulting decreased from 1.15 cm. to 0.02 cm. in height. The saline was then drawn off and normal serum introduced, four hours and five minutes after suspension. The strip immediately exhibited incoördinated contractions, and fibrillation. At the same time the strip shortened in consequence of an increase in "tone." In fifteen minutes the fibrillation disappeared and regular contractions of large amplitude began and the tone passed gradually away. The rate of contraction became slower as the contractions became more nearly normal in height, until in fifty minutes the strip remained quiet in a relaxed condition. The muscle was apparently in a state comparable to that of a fresh strip at rest in a serum bath.

After forty minutes of quiet the serum was diluted with 0.6 per cent sodium chloride solution in the ratio of one part serum to two parts sodium chloride. There was no change for sixty minutes, then contractions 1.7 cm. in height began with a very irregular rate. The contractions gradually decreased in rate and ceased in forty minutes. Six hours and twenty-five minutes from the time the serum was first used the serum-saline was further diluted to the proportion of one part serum to six parts sodium chloride. In ten minutes a perfectly regular series of contractions began, and continued for four hours. The rate during this time slowly decreased from 2 to 1.3 per minute, but was otherwise perfectly regular. The amplitude was exceptionally great, 1.7 cm. Between ten hours twenty-five minutes and sixteen hours thirty-five minutes after the serum was first introduced the strip remained for the most part quiet in diastolic state, only occasionally giving a contraction. That the above heart strip was in good condition all the time was shown by the beautiful series procured when later the strip was transferred to 0.6 per cent solution of sodium chloride.

Attention may here be called to the fact that the serum saline series of regular contractions in the above experiment extends over a time as long as or longer than that of many experiments on the frog's heart noted in literature, and that here, as in most of the experiments in this research, the experiment was continued until the after effects were fully determined. For this particular strip I have a continuous record through successive experimental conditions for seventy-three consecutive hours.

From the above examples it may be noted that automatic rhythmic contractions are developed in the ventricular strip by submitting it directly to a bath of serum diluted with a large amount of physiological saline. Also that beats are developed in a strip that has been

exposed to solutions of sodium chloride, 0.6 per cent, and then surrounded by serum. That is, it may be assumed that beats occur during that early period in the process of diffusion when the salts of the serum diffusing into the muscle mass may be assumed to be very much diluted. It must also be noted here that a strip that is quiet in normal serum may be made to beat with perfect rhythm and complete and normal amplitude, although for a variable time, simply by increasing the percentage of calcium salts in the serum.

Further, serum will revive activity in a heart strip after it has been thrown into a state of strong tone accompanied by fibrillation in consequence of the action of other solutions, such as sodium chloride solution containing calcium chloride, or a Ringer's solution used after sodium chloride. The exact type of recovery depends upon the degree of general exhaustion of the strip, *i. e.*, upon its total activity since suspension, and upon the length of time that the strip has been in fibrillation. When such a fibrillating strip is changed to serum it immediately begins to increase in activity, giving stronger and more rapid fibrillary contractions. After a longer or shorter period depending on the above mentioned conditions the strip suddenly ceases fibrillation, partially relaxes, and then begins contractions that are normal in type but irregular in rate and not frequent. (See Figs. 2 and 3.) The contractions obtained from strips that are in a more nearly exhausted state are small at first and gradually increase in height during several hours. Almost invariably, however, the contractions revived by serum become more and more infrequent, and practically cease after five to fifteen hours. This condition is again comparable to that of a fresh strip immersed in serum, except that fatigued strips, it must be remembered, have been under experimentation for several hours and have already expended a great amount of energy in muscular contractions.

Finally, a muscular strip beating for a long time in a solution of inorganic salts may ultimately gradually decrease the amplitude of its contractions and pass into a state from which it never recovers its original amplitude. When in this state it is only slightly revived by serum. This is a state of true exhaustion which will be discussed in more detail later. If such an exhausted strip be immersed in pure serum it will give a series of feeble contractions. These feeble contractions are often of quite a rapid rate, but in height do not even approximate the contraction given by the strip before exhaustion. They quickly disappear again, while serum used on a partially

exhausted strip revives quite normal contractions that persist a relatively long time. By treating the exhausted strip with the proper combination of inorganic salts a similar slight recovery in rate is produced, but never quite so great as with serum. (See experiment 42.)

Serum will revive a strip from the quiescent state — so-called exhaustion — following exposure to the action of solutions of sodium chloride or sodium chloride plus potassium chloride, such as will be described later; or from a state of tone and fibrillation caused by the use of Ringer's solution containing excess of calcium salts upon a strip exhausted by sodium chloride. In fact, serum will apparently revive a heart strip from almost any unfavorable condition brought about by isotonic solutions of the inorganic salts found in the blood, except possibly that condition of true exhaustion which has been briefly mentioned above and is described more fully in the section on exhaustion.

Sodium, Potassium, and Calcium Salts.

Sodium chloride. — Sodium chloride is the most abundant salt in the blood. Its solution in amounts isotonic with the blood has therefore during nearly thirty years been in constant use as the so-called indifferent or normal physiological saline. So far as is now known, it is the only substance that can be used in an artificial circulation medium to preserve the isotonicity with the tissues, and isotonicity seems to be a necessary factor in all experiments on artificial solutions. I have, therefore, in my experiments taken up first the effects of solutions of sodium chloride on the development and maintenance of the automatic contractions of the apex strip of the terrapin's heart.

It was shown long ago by Merunowicz,[4] 1875, that the isolated apex of the frog's ventricle would, after a certain latent period, beat automatically and rhythmically when the heart was filled with 0.6 per cent sodium chloride solution instead of with normal blood or with sheep's blood diluted with 0.6 per cent sodium chloride. Later Aubert,[8] 1881, showed that not only would the isolated apex beat when filled with sodium chloride but that it would again become quiet if the saline was replaced by undiluted blood; and further, that a heart changed back and forth would contract rhythmically or remain quiet according as it was filled with sodium chloride solution or with blood. Aubert's experiments demonstrated at least that

normal saline solution is not "indifferent" to living cardiac tissue in the same sense as is serum.

In these experiments I have tried in various ways to show what takes place when an apex strip is immersed in a bath of normal sodium chloride solution. In the earliest experiments 0.6 per cent sodium chloride was used, but later 0.7 per cent was thought to be more nearly isotonic. If a fresh strip saturated with blood be suspended in a bath of 0.6 per cent or 0.7 per cent sodium chloride solution it begins to beat rhythmically after a certain latent period. The length of the latent period, the height of the contractions, and the character of the rhythm and tone vary greatly in different experiments, but the following examples will serve as types.

TABLE I.

Table showing the great diversity of length of the latent period, rate, and maximal height of contractions produced by immersing a fresh ventricular strip of the terrapin in normal solutions of sodium chloride. The height given is the actual shortening of the strip in contraction.

Number of experiment.	Latent period before contractions begin.	Maximal height of contractions.	Rate when the rhythm first becomes regular, together with the maximal rate in some examples.
19 *b*	11 min.	1.3 cm.	12 to 14 per min.
28 *b*	1 hr. 15 "	0.15 "	3 per min.
29 *b*	1 " 30 "	0.2 "	6, increasing to 10 per min.
31 *a*	2 " 35 "	0.82 "	2.2 " " 7 " "
32 *a*	40 "	0.9 "	6 " " 9 " "
39 *c*	25 "	0.7 "	6.5 " " 10 " "
40 *c*	1 " 00 "	0.9 "	4 " " 8 " "
46 *c*	2 " 3 "	0.6 "	4 per min.

The latent period may be only a few minutes, or it may be as many hours. It seems, in general, to be shortened when the bathing solution is renewed often. In some experiments, however, it was found to be long, even though the solution was often renewed. The height of the contractions varies much in different series, although in general it may be said that the longer the latent period the shorter the contractions when they do begin, and the shorter the time they are continued. I cannot at present give any evidence showing any quantitative relation of the rate and height to the latent period. It is to be noted that a sodium-chloride series, when once established, goes

through a regular series of changes in the rate and in the height of the contractions. The rate is almost always slow and irregular at the initiation of the series, becomes regular very quickly or after an interval of as much as twenty minutes, then slightly increases in frequency while decreasing in amplitude, until the contractions are reduced to the fraction of a millimetre in height. The height of the contractions in the series is at first submaximal, quickly increases to a maximum, and then very gradually decreases to a millimetre or less, passing into what has been designated as a state of saline exhaustion, the muscle remaining quiet in a relaxed state. If the muscle is left undisturbed in the solution while it is giving the series of contractions, the series decreases in height with beautiful regularity. But if during the series the solution be renewed, a slight increase in the height of the contractions generally occurs, and this is almost always followed by an increase in rate. When the strip has become quiet in sodium chloride solution, renewal of the solution has only a very slight effect, or else no effect at all, in recovering contractions in the strip. If there is any recovery, it is never more than a minute fraction of the original amplitude. A heart strip, saturated with blood, invariably loses tone when suspended in sodium chloride solution. The loss of tone is rapid at first, but becomes less rapid as the experiment

FIG. 21. — Experiment 33-6, Feb. 3, 1898. Curve drawn by a strip of the apex of the ventricle of the terrapin suspended in one per cent sodium chloride solution. About one half the original size. Vertical magnification 4:1. Time in minutes. A latent period of fifteen minutes occurs before contractions commence. The contractions begin at a slow rate, which quickly increases to a maximum of nine to ten per minute, and remains constant during the remainder of the experiment. The height of contractions becomes maximal in fifteen minutes, then rapidly and regularly decreases to less than a millimetre after seventy minutes. It will be noted that the muscle continually loses tone during the experiment, a fact characteristic of all sodium chloride experiments.

progresses. The total loss of tone amounts to one fourth or one third the length of the strip in many experiments. In muscle strips that have previously been treated with solutions of other salts, sodium chloride produces slight or even no loss of tone.

With saline made slightly alkaline with sodium carbonate, I have never obtained a recovery of more than a small fraction of the original amplitude of the beat. This fact is noteworthy in comparison with results obtained on the saline "exhausted" frog's heart by Gaule,[7] Stiénon,[6] Martius,[9] and White.[17] According to my observations, it would seem that the heart-muscle of the terrapin treated with sodium chloride alone passes into a state from which it cannot be revived by alkaline sodium chloride solution. Although in consequence of sodium chloride treatment it gives smaller and smaller beats, and finally remains perfectly quiet in the relaxed state, still, as will be shown, the muscle strip may be thrown into most powerful rhythmic contractions at any moment, if only the proper inorganic salts be added to the saline solution. When the term "exhaustion" is used, therefore, in connection with the saline effect, it must be with the understanding that it applies only to the peculiar condition of quiescence produced by treatment with sodium chloride solution, a condition which might be designated more accurately simply as sodium chloride pause.

The amplitude of the contractions in a sodium chloride series is no criterion of the height of the contractions which the strip will give under the influence of other conditions, *i. e.*, solutions of the other inorganic constituents found in blood. In my experiments, some of the strips which I had condemned as from animals in bad condition, because they gave a poor sodium chloride series, when afterwards subjected to a bath of other inorganic salts gave contractions of unexpected amplitude. Strips cut from hearts which have previously been thoroughly irrigated with sodium chloride solution give only minute beats, or none at all, when suspended in a sodium chloride bath. They react, in fact, like the normal strip suspended in saline at the time when the saline series of contractions is nearly at an end, the exact similarity depending somewhat on the thoroughness of the irrigation. These strips, also, are not truly exhausted, as an experiment to be described later (No. 42) abundantly proves.

The great diversity of reaction to saline solutions exhibited by ventricular strips filled with blood is given at some length, because these are the variations in reactions often ascribed to the individual

peculiarities of the animal experimented upon. As a matter of fact, these reactions are only what might have been expected when we consider the cardiac rhythm in its relation to the other inorganic salts of blood. I hope later to suggest a possible explanation of these elements of difference.

If the strength of the sodium chloride to which a fresh muscle strip is subjected is varied, certain interesting phenomena are noticed. In two test experiments of three strips each, the strips in slightly hypertonic solution of sodium chloride began rhythmic contractions after a very short latent period, — a latent period shorter than was ever obtained from the fresh strips in what was assumed to be isotonic solutions, *i. e.*, 0.7 per cent. The strips in hypotonic solutions, on the other hand, were not constant in their behavior. One had an exceptionally long latent period; the other a shorter latent period than that of the control strip in isotonic solution.

TABLE II.

Variations of the strengths of the salts in relation to the latent period and to the rate and maximal height of the succeeding contractions.

Number of experiment.	Per cent of NaCl.	Latent period.	Height of contractions was reduced to 0.1 cm. in	Maximal height of contractions.	Rate when the contractions first become regular. Also the maximal rate.
35 *b*	0.7	38 min.	50 min.	0.8 cm.	8 per min. — constant.
36 *b*	0.7	24 "	70 "	0.6 "	7, increasing to 10 per min.
35 *c*	1.0	15 "	70 "	1.15 "	9.5 per min.
36 *c*	1.0	3 "	42 "	1.0 "	9, increasing to 11 per min.
35 *a*	0.4	3 "	42 "	0.7 "	5 " " 8 " "
36 *a*	0.4	44 "	60 "	0.5 "	6 " " 9 " "

The general cycle of events described above is characteristic of the saline curve of the normal strip. But if the strip is first treated with some other solution or kept in terrapin serum or blood for two or three days, or has been revived from a previous saline treatment and then again subjected to saline solution, its reactions present certain characteristic differences. Figures 2 and 3 illustrate these.

Figure 2 gives the reactions of two strips from the apex of the ventricle of the terrapin, the two from the same heart. Trace *a* begins at *x* with the immersion of the strip in serum twenty-one hours after suspension and following an unfavorable Ringer's solution.

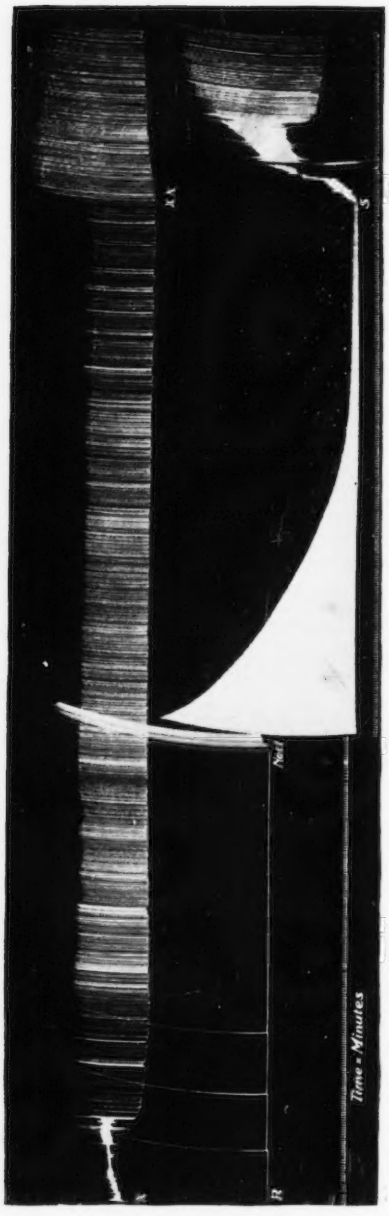

FIGURE 2. Experiment 30*a* and *b*, January 18, 1898. Vertical magnification, 4 : 1. Time in minutes. Reaction of two strips from the apex of the ventricle of a terrapin. *Trace a*. At $\frac{1}{2}$, immersion in serum; *xx*, the serum drawn off and Ringer's solution (NaCl 0.6%, $CaCl_2$ 0.026%, KCl 0.04%, Na_2CO_3 0.003%) substituted. *Trace b*. A strip — quiet and relaxed in dilute serum, after 21 hours' suspension — was immersed at *R* in Ringer's solution (NaCl 0.6%, $CaCl_2$ 0.026%, KCl 0.05%, Na_2CO_3 0.003%). At NaCl sodium chloride 0.6% substituted for the Ringer's solution (shortly afterward it was necessary to alter the position of the writing point.) *S*, immersion in dilute serum. The curve is about one half the original size.

The serum immediately produced fibrillation and tone increase, but after thirty-five minutes the strip suddenly relaxed and developed normal contractions at an irregular rate. The height remained nearly constant for seven hours. At *xx* the serum was drawn off and Ringer's solution (NaCl 0.6%, $CaCl_2$ 0.026%, KCl 0.04%, and Na_2CO_3 0.003%) substituted. This was followed by a strong increase in the amplitude of contractions and a slight acceleration of rate. Trace *b* was recorded after a suspension of twenty-one hours. The strip was quiet and relaxed in dilute serum. At *R* it was immersed in Ringer's solution (NaCl 0.6%, $CaCl_2$ 0.026%, KCl 0.05%, and Na_2CO_3 0.003%). No change was produced by the solution for three hours and ten minutes. The three isolated contractions indicate the state of the

muscle. A change to 0.6 per cent NaCl was immediately followed by a rapid rhythm, eight and more per minute. After four hours in sodium chloride solution the contractions were reduced to a small fraction of the original amplitude. That the activity of the strip was only suspended is shown by the effect of the dilute serum which was used at *S*. Serum produced strong increase in tone with fibrillation, which passed off gradually into well-coördinated contractions. Figure 3 gives the change in the experimental conditions following the effects shown in Fig. 2. In this experiment after thirty hours' suspension the strips *a* and *b* from terrapin No. 30 were immersed in 0.64 per cent sodium chloride solution; *a* had been previously immersed in Ringer's solution, *b* in serum diluted with 0.6 per cent sodium chloride. Strip

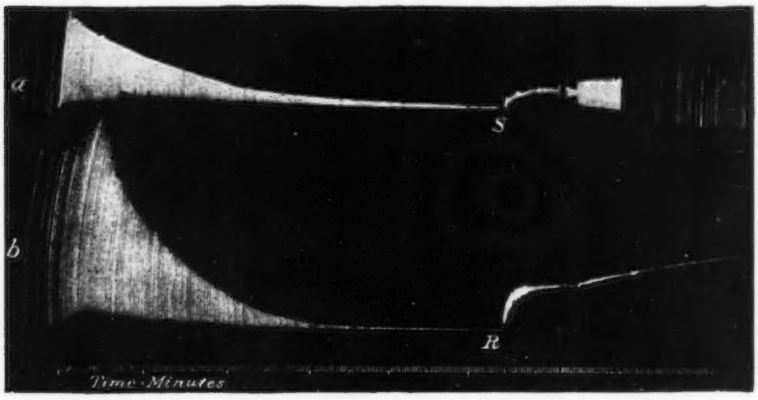

FIGURE 3. Experiment 30, *a* and *b*, January 18, 1898; vertical magnification 2/1; after thirty hours' suspension. Curves drawn by strips from terrapin ventricle immersed in 0.64 per cent sodium chloride solution. Strip *a* had been previously immersed in Ringer's solution; strip *b* in serum diluted with 0.6 per cent sodium chloride. *S*, immersion in serum; *R*, immersion in Ringer's solution. One half the original size.

a contracted at the rate of eleven to twelve and *b* at the rate of seven per minute. The similarity between these two traces and the corresponding one in Fig. 2 is very striking and suggestive. After two hours forty minutes in sodium chloride solution, serum produced in *a* an increase in tone with fibrillation, followed by good contractions. Ringer's solution produced in *b* a marked increase in tone with incoördinated contractions and fibrillations, from which the muscle did not recover until serum was again used.

First and perhaps most important of the characteristic differences illustrated by Figs. 2 and 3 is the shortening or entire absence of the latent period. If the strip has already been submitted to several changes and is still in condition to contract, then saline solution calls forth immediate contractions. Under these conditions also the contractions begin at a submaximal height, usually that of the contractions in the preceding solution, quickly increase to a maximum, and then regularly and uniformly decrease to complete disappearance, the muscle remaining inactive in a relaxed state. In these cases the maximal sodium chloride contractions are maximal for the muscle under *any* condition, a fact in sharp contrast to the submaximal contractions so often obtained from a fresh strip treated with sodium chloride solution. The rate is also much increased, in many experiments reaching twenty or more per minute, a rate too rapid to be distinguished in the records made on the slowly moving drum. The effects present the appearance of a very much heightened irritability, and the longer the muscle has been suspended the more striking is the result.

The rate in sodium chloride remains more nearly constant than in any other solution except, perhaps, very much diluted blood or serum. The decrease in the height of the contractions brought out in a heart strip by a second sodium chloride treatment is almost always perfectly regular and symmetrical. Such regularity in the response of the muscle to a definite salt in solution argues for a definite and regular series of changes in the muscle, either of a physical, *i. e.* osmotic, or of a chemical nature. I shall only briefly call attention here to certain facts which are brought out by this particular group of experiments.

It must be borne in mind that the muscle strip cut from a fresh unwashed ventricle is full of blood, that is, its muscle fibres are bathed in a liquid containing the numerous constituents of blood. When this strip is suspended in a solution of sodium chloride as nearly isotonic with blood as may be, it is to be presumed that diffusion of the salts other than sodium chloride immediately begins. Presumably osmotic currents of water and the diffusion of sodium chloride are reduced to a minimum, but all other diffusible constituents of the blood will tend to pass into the surrounding liquid. The more frequently this surrounding liquid is renewed the more rapidly the process is carried on. When contractions begin, as they do sooner or later, the rhythmical change in pressure greatly

aids the process. During the first few contractions the blood is squeezed out of the interstices of the strip in quantities sufficient to discolor the saline solution. When the whole heart is irrigated by saline solution before the strips of ventricle are cut the same process must necessarily go on during irrigation. In this case it is much more rapid, since the saline solution flows through the coronary system of blood vessels. The heart always contracts when irrigated, hence this mechanical aid to the washing out process is most effective in irrigating the whole heart.

Recalling, then, in the first place, that the apex ventricular strip cut from a heart previously irrigated and washed with saline solution will not contract when suspended in a saline solution, and secondly, that a heart strip filled with blood gives a rhythmic series of beats, diminishing rapidly to zero when suspended in a saline solution, and thirdly, that a strip revived by other solutions and again surrounded by sodium chloride solution repeats essentially the same process, my results may be briefly summarized as follows:

1. Sodium chloride in solution in distilled water will sustain the ventricular muscle of the terrapin in rhythmic contractions for a brief time only, one to two hours.

2. The ventricular muscle is not exhausted by sodium chloride solution, although the decreasing amplitude of the series of contractions simulates exhaustion.

3. Sodium chloride solutions stimulate a heart strip to a brief series of contractions of increasing rhythm and decreasing amplitude.

4. A sodium chloride bath does not always develop maximal contractions in a heart strip.

5. Sodium chloride solution produces marked loss of tone in a fresh ventricular strip.

Calcium chloride.—Calcium exists in the blood presumably in combination and as free salts or the corresponding ions. Quantitative analyses of the calcium present in the blood of the terrapin were made in several instances. A large terrapin was bled for such an analysis, and the blood kept on ice a day or more until the corpuscles had settled. From fifty to sixty cubic centimetres of clear amber-colored plasma were obtained from a single terrapin. The plasma to be analyzed was siphoned off into a standardized graduated burette, measured amounts drawn into large centrifugalizing tubes, ammonia added to slight excess, and the calcium precipitated with ammonium oxalate. The precipitate was now left to settle, or else

was thrown down by means of a centrifugal machine, washed in distilled water, redissolved in weak hydrochloric acid, reprecipitated, washed until free of chlorine, dried, and finally heated in a platinum crucible until the weight remained constant. Duplicate analyses were made and the results are expressed as grams of calcium oxide in 100 c.c. of plasma. Each tube in Experiment I contained 24 c.c., in Experiment II, 25 c.c. of plasma.

TABLE III.

Determination of calcium in the plasma of the slider terrapin expressed as calcium oxide in 100 c.c. of plasma.

Experiment I. December 4, 1897. $a = 0.0126$ gram, $b = 0.0126$ gram.

Experiment II. December 11, 1887. $a = 0.0133$ gram, $b = 0.0141$ gram.

Mean of four determinations, 0.0131 gram.

This determination is in fair agreement with the results of Gerlach,[20] secured by the same method from dog's serum, namely, 0.014 and 0.0145 gram CaO in 100 c.c. serum, and also with determinations of the calcium in sheep serum made by Dr. Howell in this laboratory by a volumetric method with potassium permanganate, which gave as the mean of two analyses 0.0124 CaO in 100 c.c. of serum.

The mean of the determinations in Table III expressed as calcium chloride is 0.026 gram per 100 c.c. of plasma. This amount was considered as the amount normal to the blood. Chloride salts were used in all the artificial inorganic salt solutions in order to reduce the effect of the acid radical to a constant. One would expect the amount of calcium to vary in different individuals. It is possible, too, that some of the combined calcium may separate off, and if so the above amount would be too large. It will, however, serve as the most available constant in the study of the effects of the calcium in the blood in its relation to the development and maintenance of contractions in the isolated heart strip. In 1883 Ringer[10] first demonstrated the great importance of this element in the activity of the frog's heart. Since that time numerous experimenters have extended and enlarged our knowledge of the physiological importance of calcium in the animal organism. At the present time it is generally recognized that calcium in some form plays an essential part not only in the activity of muscle but also in the clotting of blood, the coagulation of milk, etc.

Ringer demonstrated that calcium does not act alone in maintain-

ing the rhythm of the frog's heart, but that it must be antagonized by potassium salts. I will here first discuss briefly the effects of calcium and potassium salts as such, and later take up their relation to other salts. Isotonic strengths of calcium are imperfectly borne by the cardiac muscle, hence calcium effects must be studied in combination with some other isotonic solution. Of these I have used isotonic solutions of sodium chloride, dextrose, and urea. The last seems injurious, and no contractions have been observed in it. My most reliable calcium effects are, therefore, those obtained in combination with saline effects.

Calcium chloride alone. — Calcium chloride in distilled water in approximately isotonic solution when applied directly to a heart strip throws the muscle into strong tone. No rhythmical contractions are given off for five minutes, — the longest time a strip has been submitted to this excessively strong solution. When the excess of calcium is removed by washing the strip with 0.7 per cent sodium chloride solution, a series of very rapid contractions immediately starts up. The rhythmic contractions are superposed upon a state of strong tonic shortening. No permanent injurious effect follows the use of the strong calcium solutions. If, on the other hand, calcium chloride of the strength found in the blood is applied to a heart strip that is quiet but in good condition for contracting, its application is followed immediately by a rapid and regular series of contractions. The contractions decrease rapidly in height for five minutes, — the longest time a strip has been submitted to this hypotonic solution. In these contractions the relaxation phase is much shortened and the lever does not return to the base line.

The experiments with solutions of calcium chloride alone are not very satisfactory although suggestive. Isotonic solutions on the one hand contain a deleterious amount of calcium, and on the other hand normal strengths of calcium are so strongly hypotonic to the muscle that this physical factor is doubtless a predominant one in determining the results.

Potassium chloride. — Potassium chloride, like calcium chloride, is deleterious when applied to the muscle of the heart in isotonic solutions. Its effects are also complicated by physical phenomena when it is applied in solutions of a strength found normally in the blood. When one per cent potassium chloride solution was applied to a heart strip which was previously contracting rhythmically in dilute serum the heart strip quickly gave one or two spasmodic contractions

and then remained quiet in a condition of tone. Afterward, when the excess of potassium was removed by washing the strip with 0.7 per cent sodium chloride, no contractions were developed but the tone spasm passed off. Dilute serum again established a rhythm after a short latent period, and the rhythm appeared perfectly normal in character. From this it will be seen that excessive doses of potassium as well as of calcium salts do not produce a permanent poisonous effect on the ventricular strip when applied for short periods.

In more dilute solutions, *i. e.*, in solutions approximately normal to the blood (0.03 to 0.04 per cent), as determined by physiological reaction and by analyses reported for the blood of other animals, potassium chloride applied to a contracting strip produced quiescence after a few contractions which decreased rapidly in amplitude to complete disappearance. Tonic shortening also followed the use of this hypotonic solution.

Sodium, Potassium, and Calcium Salts in Combination.

Calcium chloride in isotonic solutions of sodium chloride. — If calcium chloride, in an amount normal or subnormal to the blood, be added to isotonic solutions of sodium chloride, certain important results are obtained. This mixture of salts, applied to a fresh, unwashed heart strip, produces a series of contractions after a very short latent period. The series resembles, in general features, the series of contractions given by a control strip in saline alone. But the rate is more rapid in the strip in sodium and calcium chloride solution, and the contractions are maximal from the first, while in the pure sodium chloride solution contractions are usually not maximal for some minutes after the series is inaugurated. The most characteristic effect of the calcium when added to sodium chloride solution is the prevention of perfect relaxation after each contraction. There is a strong rise of the base line instead of the gradual fall so characteristic of the sodium chloride series. If the amount of calcium chloride used on a fresh strip is large, say 0.04 per cent, the rise occurs very soon after the strip begins automatic contractions. The rise begins immediately on the application of the solution when applied to a strip that is quiet after a previous sodium chloride treatment. When a strip ceases to beat in a sodium and calcium chloride mixture it ceases in a state of tone. If the amount of calcium

is small, 0.01 per cent, then the increase in muscular tone is only slight. Calcium chloride in solutions of sodium chloride never more than slightly revives a heart strip after it has ceased to contract in a solution of sodium chloride alone.

Calcium chloride in isotonic solutions of dextrose. — Calcium chloride in amount normal to the blood, 0.026 per cent, when applied in isotonic solutions of dextrose to a heart strip previously beating in serum, or in serum diluted with saline, immediately calls forth a series of rapid contractions, together with a strong increase in tone. When the dextrose alone is applied to the heart strip, it calls forth a similar, though less rapid, series of contractions. It is questionable, therefore, just how much of the above effect is due to dextrose and how much to calcium, a point which requires further investigation.

Calcium chloride in isotonic solutions of urea. — Calcium chloride in isotonic solutions of urea produced no contractions in the muscle strip. Urea itself seems injurious to cardiac muscle when applied in isotonic strength, for even serum produces only slight or no recovery after its use.

Potassium chloride in isotonic solutions of sodium chloride. — When a fresh muscle strip is immersed in a solution of 0.03 per cent potassium chloride in isotonic solutions of sodium chloride, either no contractions at all are developed, or, if developed, the contractions are extremely minute, and make their appearance only after an extremely long latent period. The contractions recorded in the exceptional cases occur very irregularly, and are only a small fraction of the height (one ninth in one experiment) of the contractions given after a change to a solution of different composition. These results are in sharp contrast with the behavior of a strip surrounded by sodium chloride alone, or by sodium and calcium chloride solution. Strips in solutions of sodium and potassium chloride almost always show an excessive loss of tone, a result just the opposite to that of strips in solutions of sodium and calcium chloride. Solutions of sodium and potassium chloride have no effect in reviving activity in a strip that has ceased to beat in sodium chloride alone.

Ringer[10] and his students have taken a prominent part in investigations concerning the action of potassium salts on the animal body and on the heart. They have shown that potassium salts applied to the frog's heart produce a slowing of the rate, much dilatation,

and ultimate cessation of the rhythm. Ringer was also the first to show the necessity of this salt in antagonizing the excessive stimulating effect of calcium salts. We will now turn to this phase of the subject as applied to the terrapin heart strip.

Sodium, calcium, and potassium chlorides in isotonic solution. — The wonderful sustaining power of sodium, potassium, and calcium salts in solution was first pointed out by Ringer[10] in 1883. In the proportions used in his original formula the solution was shown to revive the frog's heart, and to sustain it in rhythmic contraction for several hours. Ringer afterward substituted the tribasic phosphate for the calcium chloride; but, in so far as the cardiac muscular strip is concerned, I have obtained quite satisfactory results with the chloride.

In my experiments I have striven to secure a proportion among the above inorganic salts that would give the effects on an isolated cardiac strip most nearly approaching that of blood. The facts already pointed out by Ringer and his students, and by Howell and Cooke,[15] seem to indicate that if one could exactly imitate the composition of the blood as regards the inorganic constituents, it would be possible to secure, approximately, the same effects from blood and from the artificial preparation of inorganic salts in so far as the isolated strip is concerned.

By keeping the amount of calcium in isotonic sodium chloride solution constant and equal to the mean of the two analyses of the terrapin plasma, and by varying the amount of potassium, a solution was soon determined which gave fairly constant results. With this solution results were obtained that closely approximated the effects of a serum bath on the ventricular strip. The amounts of the three salts in this solution were: sodium chloride, 0.6–0.7 per cent; calcium chloride, 0.026 per cent; potassium chloride, 0.03–0.04 per cent; a trace of sodium carbonate was sometimes added. This solution of the inorganic salts of blood will keep a heart strip alive and in condition to beat for a very long time, 72 hours and more. The inorganic solution does not keep the strip in as good condition as does blood or serum, but the parallelism between the two is very striking. The following experiment exhibits the close relation in the action of the above inorganic salt solution and of serum on ventricular strips.

Experiment 34, January 29, 1898. The apical third of the ventricle was cut into thin strips which were suspended in heart tubes while still saturated with

blood. Strip *a* was immersed in pure serum; strip *d* in a bath of Ringer's mixture (0.6% NaCl, 0.026 $CaCl_2$, 0.04% KCl, and a trace of Na_2CO_3).

a. The serum strip gave a few beats when first suspended, but in a few minutes became quiet in a relaxed state. Single contractions occurred at long and irregular intervals during ninety-six hours, when the strip was changed to a bath of 0.6 per cent sodium chloride. Sodium chloride solution called forth the customary series of contractions.

d. The strip in Ringer's solution remained quiet in a relaxed state, giving only occasional single contractions during seventy-six hours. The contractions which were recorded during a portion of this time did not average more than one per hour. At the end of seventy-six hours the strip was changed to a bath of 0.6 per cent sodium chloride solution, and a rapid series of contractions was produced at once (see Fig. 4). While the maximal contractions of this series were only 0.5 cm. high, about one half the height of those of strip *a* in saline, still the series resembled a typical sodium chloride series in that the contractions rapidly declined in amplitude until the strip remained quiet in the diastolic state.

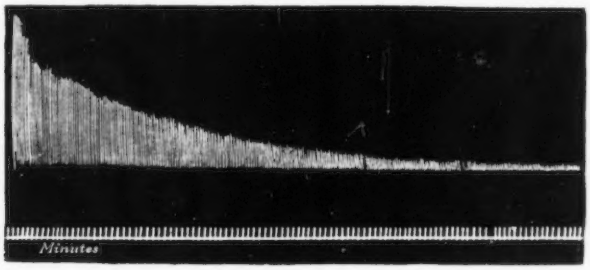

FIGURE 4. Experiment 34 *d*, January 29, 1898. Vertical magnification 4/1. Time-trace marks minutes. To illustrate the effect of a 0.6 per cent bath of sodium chloride solution on a strip of terrapin's ventricle after seventy-six hours' suspension in Ringer's solution. When the sodium chloride was introduced, regular contractions began at once, as the above shows.

The following is a second example of the similarity of action between the solution of inorganic salts and pure serum: —

Terrapin 45, February 28, 1898. The apex of the ventricle saturated with blood was cut into thin strips and suspended in heart tubes, *a* in Ringer's solution, *b* and *c* in air. Strip *b* was moistened by occasional drops of Ringer's solution (0.7% NaCl, 0.02% $CaCl_2$, and 0.03% KCl), and *c* with drops of serum.

Strip *a*, after a latent period of twenty minutes, gave contractions that were

perfectly normal in character but very irregular in rate. The rate averaged one to four per minute. Neither *b* nor *c* contracted for thirty consecutive hours, but when moistened with sodium chloride solution both strips gave contractions that were complete and apparently normal in character.

This experiment again demonstrates that solutions of inorganic salts, as well as serum, are able to keep the ventricular strip in good condition, and that they, like serum, will not necessarily stimulate the strip to rhythmic contractions.

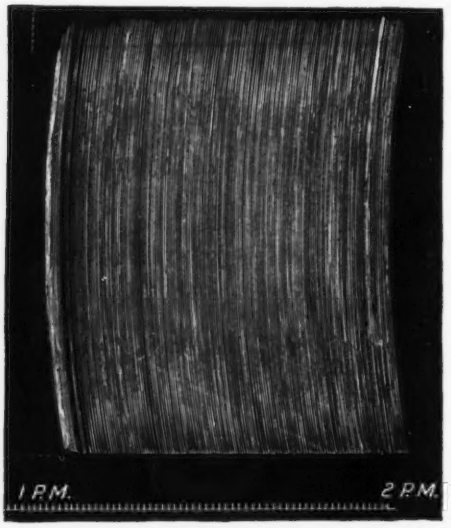

FIGURE 5. Experiment 42 *b*, February 14, 1898. Vertical magnification 4 1; time trace marks minutes. A ventricular strip from the apex of a terrapin's heart, moist with Ringer's solution, and contracting in a moist chamber. See protocol of Experiment 42. This strip, at the end of twenty-two hours' suspension in air, and still moist with Ringer's solution containing 0.7% NaCl, 0.026% $CaCl_2$, and 0.03% KCl, contracted only at long intervals, giving only sixteen contractions in the last two hours. When the strip was moistened with Ringer's solution, with the calcium chloride increased to 0.04%, the above beautifuliy regular contractions began and continued for one hour fifty minutes without interruption. Rate, four per minute. Compare with Fig. 6.

This experiment is also important in its bearing on another experiment to be given presently, Experiment 42. The fact that one heart strip immersed in a given inorganic solution beats while another strip from the same heart does not beat when in air saturated with water vapor, and moistened only occasionally with the same inorganic solution, indicates some process more favorable to the development of contractions in the presence of the liquid bath. This cannot be ascribed to any hindering effect due to the presence of the air. In fact, one would expect the better aëration of the tissue surrounded by air to facilitate the development of contractions rather than to delay contractions. This point is brought forward and emphasized at this

time; for if strips be first washed out in sodium chloride solution, then bathed for a few minutes with Ringer's solution, and suspended in air, they may become as much as ten times more active than strips kept in a constant bath of the same solution.

The particular results to be expected when a fresh strip of ventricle containing the normal amount of blood is subjected to a bath of sodium, calcium and potassium chloride solution, so far as my experiments go, depend upon the relative proportions of the calcium and potassium chlorides. If these salts are in the proportions of 0.026 per cent calcium chloride to 0.03 per cent potassium chloride, a few good contractions at a very slow and irregular rate may result. If this ratio is changed by increasing the calcium or by decreasing the potassium, then the contractions are increased in frequency (Figs. 5 and 6). But if the calcium is diminished or the potassium increased, few contractions are developed, or none at all. Experiment 34 *d* gave fifteen contractions in the first forty-six hours. The solution used contained 0.6 per cent sodium chloride, 0.026 per cent calcium chloride, 0.04 per cent potassium chloride, and 0.003 per cent sodium carbonate.

If the muscle strip is cut from a heart previously irrigated with saline, or from one that has been subjected to the influence of some other artificial solution, the effect produced on the strip by Ringer's solution varies according to at least two factors: first the relative amounts of the calcium and potassium chlorides in the Ringer's solution, and second, the composition of the solution to which the strip has previously been submitted, together with the time the latter has acted.

Ringer's solution following sodium chloride solution. — A ventricular strip treated with sodium chloride, 0.6 to 0.7 per cent, until it ceases to contract, is stimulated to a strong and regular series of contractions when bathed in a suitable Ringer's solution. The amplitude and general character of the contractions of a strip revived in this way can only be compared with the contractions of a strip revived by serum or by serum diluted with saline. By moistening the strips with Ringer's solution, in which the proportion of calcium chloride is increased from time to time, strips may be kept beating for sixty hours and more after the apparent exhaustion in sodium chloride. In one experiment, No. 42, three companion strips were in this way kept contracting for fifty-one and a half, sixty-two, and forty-two hours.

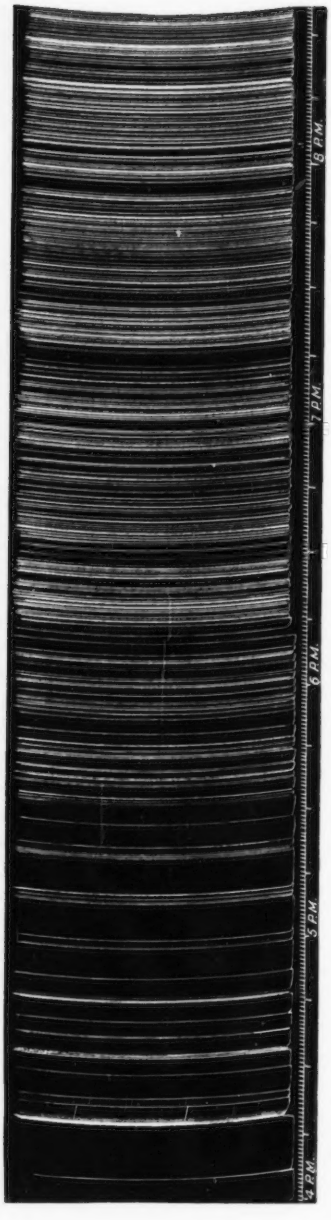

FIGURE 6. Experiment 42 *a*, February 14, 1898. Vertical magnification 4/1. Time tracé in minutes. To illustrate the effect on a quiet strip of increasing the amount of calcium chloride in the Ringer's solution used. The curves are about one half the original size.

The ventricular strip from which this trace was obtained was from a terrapin's heart that had been washed free of blood with 0.6 per cent sodium chloride solution, then suspended in a Ringer's solution until a strong rhythm was established, and finally left contracting in moist air. The contractions were always full, but the rate became slower and more irregular, and ceased at eighteen hours after suspension. The strip was now moistened with stock Ringer's solution, then with a solution containing successively 0.03%, 0.04% and 0.05% and finally 0.06% calcium chloride. The last moistening occurred at the twenty-seventh hour of suspension. In twelve minutes after the last moistening contractions began again at an irregular rate. The contractions increased in frequency, and became fairly regular in four hours, as the above trace illustrates. See also Fig. 5.

In another experiment, 33 *a* and *d*, strips immersed in Ringer's solution contracted rhythmically and normally for twenty-seven hours, when the record was lost. After forty-two hours these strips were quiet, but apparently in good condition for contraction, as both strips were immediately revived, and gave fine contractions when changed to serum. The recovery in both these strips in serum closely resembles the recovery of contractions in strips treated with sodium chloride, and then changed to Ringer's solution or to extract of serum. This suggests a recovery due to the effects of the better combination of salts in the terrapin's own serum. At least, I am strongly inclined to believe that the revival in this case is due to the fact that serum contains the conditions or sub-

stances most favorable to the complete using up of the contractile material still in the strip.

If the strip is changed from sodium chloride to Ringer's solution while the contractions are small and regular in rate, the revival of complete contractions is very prompt. The contractions become maximal in a very few minutes, and the rate is often perfectly regular for a time. Afterward, however, the rate becomes slower and very irregular, a result perfectly analogous to the serum effect under the same circumstances.

On the other hand, if the contractions have entirely ceased in sodium chloride, and the muscle has remained in the solution from one to several hours, and then is suddenly subjected to a bath of Ringer's solution, the result is quite different. Under such circumstances the muscle is thrown into immediate fibrillation, from which it never recovers while in the Ringer's solution. This is apparently strictly a calcium effect. For if, instead of the ordinary combination of salts in the Ringer's solution, a solution containing a reduced amount of calcium is first applied to the strip and the calcium gradually increased to the normal amount, good contractions are produced and fibrillation avoided.

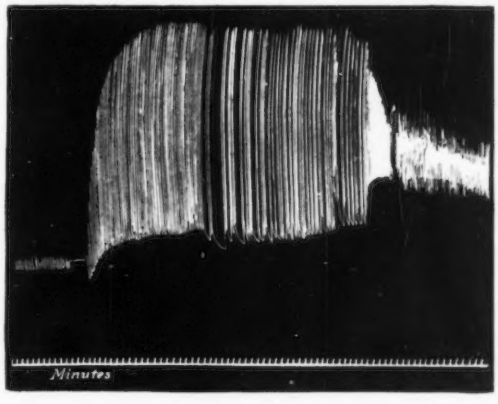

FIGURE 7. Experiment 46 *a*. March 2, 1898. Vertical magnification, 4 : 1. Time trace marks minutes. To illustrate the effect of a favorable combination of the salts in Ringer's solution in reviving contractions in a strip of the terrapin ventricle after its contractions in sodium chloride were reduced to a fraction of a millimetre in height. On immersing the strip of ventricle in Ringer's solution the contractions quickly increase in amplitude to a maximum, become irregular in rate after twenty minutes, and pass into fibrillation after about fifty minutes. Seven ninths the original size.

Muscle strips left in sodium chloride solution a long time invariably go into strong contracture when changed to ordinary Ringer's solution, whether they beat or not. If fibrillation results, the tonic shortening amounts to one third or even one half the amount of

shortening previously produced in the same muscle during a normal contraction. If the saline strip is treated with a solution of sodium chloride and potassium chloride before the Ringer's solution is used, contracture is diminished and fibrillation delayed, though not necessarily avoided.

In certain experiments normal Ringer's solution following sodium chloride solution produced an excellent recovery for a time, but later the strips went into a state of fibrillation. Perhaps it would be better to describe this state as one of incoördinated contractions followed by fibrillation. Such strips while giving regular normal contractions in Ringer's solution suddenly show independent rhythm in two or three parts of the strip at the same time, and then gradually pass into fibrillation.

In a single example, a strip contracting in serum after repeated revivals from sodium chloride solution gave much larger beats when changed from serum to Ringer's solution (see Fig. 2 *a*). The height increased from 0.6 cm. to 1.05 cm. The rate, which was irregular both before and after the change, was increased from an average of 0.3 to 2.8 per minute. This effect followed at once, and is presumably due to the stimulating effect of the calcium.

Ringer's solution following a solution of sodium and potassium chloride. — A normal strip that has been kept from contracting by a solution of potassium in sodium chloride gives normal rhythmic contractions when the bath is changed to Ringer's solution. If the amount of potassium chloride in the sodium and potassium solution has been great, say 0.04 to 0.05 per cent, the contractions called forth by the bath of Ringer's solution may be at a very slow rate, or may appear in groups at a good rate, but with the groups separated by long periods of rest, during which the muscle remains in a perfectly relaxed state. Ringer's solution after a solution of sodium and potassium chloride is not followed by tone shortening, such as occurs after sodium chloride alone.

If a strip in a sodium and potassium chloride solution be transferred first to a Ringer's solution having a reduced amount of calcium, and later the calcium be increased, no contractions are developed until the amount of calcium in the Ringer's solution is increased to at least the normal amount found in blood.

Of the strips which were transferred to a bath of ordinary Ringer's solution after treatment with a solution of sodium and

potassium chloride, two developed full normal contractions in from five to ten minutes, and after forty to sixty minutes each strip fibrillated. Presumably this result was due, in each case, to the strong stimulating effect of the calcium, which was fully counteracted by the potassium until the calcium had time to diffuse more completely into the strip.

Extract of the Evaporated Residue of Serum.

Terrapin serum was dried over a water bath, and the dried residue pulverized and extracted with distilled water. The filtered extract was evaporated to dryness and a second extract made and diluted to the original volume of serum. The second extract gave

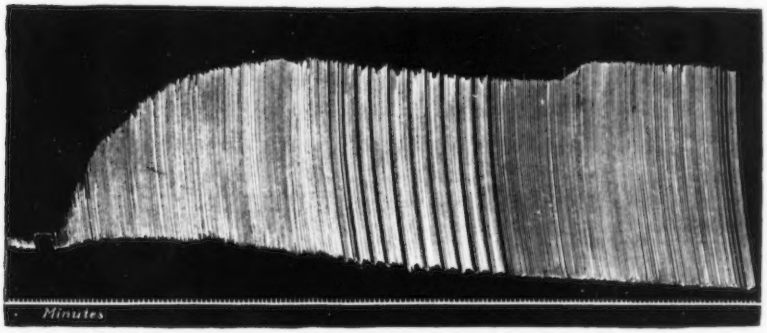

FIGURE 8. From Experiment 46*c*, March 2, 1898; vertical magnification 4 : 1. Time trace marks minutes. To illustrate the effect of an extract of the dried residue of terrapin serum in reviving full contractions in a strip of ventricle that had almost ceased to contract in sodium chloride solution. Companion to the tracing shown in Fig. 7.

only a very faint color change when tested for proteid by the xanthoproteic test, and no proteid was detected by Millon's reagent. Careful testing for sugar with Fehling's fluid gave a questionable precipitate. This extract was compared with blood and with Ringer's solution in its reviving effects on the heart strip after saline treatment. The close similarity in the results is shown in the following protocol and the accompanying Fig. 8.

Experiment 46, March 2, 1898. Three ventricular strips cut from the normal heart were first immersed in saline until the contractions produced in each were reduced to 0.04 cm. and less in height. Strip *a* was transferred

from saline to Ringer's solution (0.7% NaCl, 0.026% $CaCl_2$, 0.03% KCl). Strip *b* was transferred to serum, and *c* to an extract of the salts from the evaporated residue of serum.

Strip a. So soon as Ringer's solution surrounded the strip, contractions began and continued at a regular rate but rapidly increased in height with each successive contraction until in ten minutes they had increased from 0.04 cm. to 0.8 cm. The rate remained regular for twenty minutes, then became irregular for thirty minutes. After fifty minutes the strip gave a series of very rapid contractions and then went into fibrillation, from which it did not recover. (Fig. 7.)

Strip b. In serum; was accidentally lost.

Strip c. The extract of the salts of serum produced a rapid recovery of the amplitude, but not so rapid a recovery as that of strip *a* in Ringer's solution. The height increased in thirty minutes from 0.03 cm. to 0.9 cm. The rate for the first hour was slightly irregular, being from four to five per minute. After one hour in serum extract Luciani's periods appeared. There were ten of these groups in forty minutes. In every group the contractions began at a slow rate, increased to a maximum rate, then became slow again. A pause of from thirty to sixty seconds intervened between each two of the several groups. The rate following the groups was perfectly regular — two per minute — for several minutes. At the end of two hours and thirty-five minutes the contractions suddenly ceased entirely. With the exception of three single contractions the strip remained quiet in relaxation until transferred to 0.7 per cent saline, when it gave the customary saline series. The last contractions of the serum extract series were 1.03 cm. high. This series ended very much like that of a slightly diluted serum.

On the whole, the serum extract revived the strip *c* more normally, that is, more like serum, than did Ringer's solution in the case of strip *a*. The after effects were also more like serum, as shown in other serum experiments. Another experiment with serum extract, on strip *a* of Exp. 46, bears out this view. Serum extract was used on the strip when in fibrillation after long exhaustion, and at once produced increased activity. After seventy minutes the fibrillating strip relaxed suddenly, and began slow and irregular, yet apparently perfect contractions. The relaxation was, however, only partial in this case. This was the only instance in all my experiments in which I obtained this type of recovery from fibrillation with anything but pure serum.

On the Work given off by a Cardiac Strip and the Source of the Energy of Contraction.

The amount of energy liberated by a heart strip bathed in an inorganic salt solution has been so great in many experiments as to raise the question as to the source of the energy-giving material.

In Experiment 33 *a* a fresh strip that had been bathed in 0.6 per cent sodium chloride until no further beats were produced was changed to a bath of Ringer's solution. It almost immediately gave contractions which were normal in general character, and higher and stronger than the largest contractions obtained from the strip while in the sodium chloride bath. The rate, however, was slow and irregular. The contractions of this strip in Ringer's solution increased in height and improved in rate, and in twenty-seven hours gave 1670 gramcentimetres of work. The record was lost from the twenty-seventh till the forty-second hour, at the end of which time the quiet muscle was transferred to serum. This partial record strongly suggests the view that the amount of energy developed by the muscle strip is greater than can be accounted for by the proteid constituents of the blood in the muscle. To test this question more thoroughly, Experiment 42, given in detail below, was tried.

Experiment 42, February 14, 1898. A terrapin's heart was washed as free as possible of blood by continuous irrigation with 0.7 per cent sodium chloride solution. The washing was facilitated by cutting the coronary veins and by gentle massage applied to the ventricle. Irrigation continued one hour, and at the end of that time the ventricle was still giving feeble contractions. Thirty minutes were consumed in preparing, weighing, and suspending three ventricular strips. They were, therefore, bathed in 0.7 per cent solution of sodium chloride an hour and thirty minutes. The strips in the heart tubes were next surrounded by a bath of Ringer's solution (0.7% NaCl, 0.026% $CaCl_2$, and 0.03% KCl) for thirty minutes, during which time strong contractions were established in each strip. The Ringer's solution was next drawn off, leaving the strips in moist air. They were occasionally moistened with a momentary bath or with drops of Ringer's solution. The three strips were kept thus in air fifty-one and a half, seventy-two and two thirds, and fifty hours respectively.

Strip a. This strip was kept moistened with Ringer's solution, of the composition given above, for seventeen hours twenty-five minutes. The contractions were comparatively rapid at first, about one half the total number of

contractions occurring during the first seven hours. During this first period of suspension the rate slowly decreased, so that at the end of eight hours the strip was beating with an irregular rhythm and with a tendency to grouping. The rate temporarily increased after each remoistening of the strip. The amplitude began with 0.9 cm. actual shortening during a contraction, and slowly but steadily increased to 1.38 cm. in twenty-six hours; it remained above 1.0 cm. for forty-five hours. The contractions were apparently always complete whether the rate was fast or slow. The record was lost from thirty minutes past the tenth hour to fifteen minutes past the seventeenth hour of suspension in air. At the end of this time strip *a* was not contracting. Remoistening with the usual Ringer's solution produced no contractions. Ringer's solution with increased amounts of calcium chloride was then applied to the strip. During eight hours (the seventeenth to the twenty-fifth) the calcium chloride was increased successively to 0.03, 0.04, 0.05, and 0.06 per cent. With Ringer's solution containing 0.06 per cent calcium chloride, the strip again began to contract and continued to do so for twenty-six hours thirty minutes, with but one remoistening. The contractions, at first slow and irregular in rate, increased from forty the first hour (twenty-sixth of the experiment) to one hundred and sixty-one the sixth hour (thirty-second of the experiment). During the succeeding night the room temperature decreased to 4° C. and both rate and height of contractions were somewhat reduced, but both recovered again the next morning.

The forty-fifth hour after suspension in air the contractions began slowly to decrease in amplitude, reaching a zero in six hours, thirty minutes, *i. e.*, after a total time of activity in Ringer's solution of fifty-one hours and thirty minutes. The proportion of calcium chloride was again increased, but no contractions were produced even with 0.10 per cent.

A bath of horse's serum applied to the strip at fifty-one and a half hours was followed immediately by a series of contractions lasting for sixteen hours. The rate began with five per minute, but varied greatly at different times and the contractions were often quite incoördinated. In serum the height was 0.35 cm. at first, about one third that of the normal contractions of this strip, but decreased to zero in sixteen hours, *i. e.*, the sixty-seventh hour of the experiment. Minute contractions, 0.02 cm., were still obtained by diluted serum at the seventy-fourth hour.

Strip b. This strip was continued in air for seventy-one hours. For the first ten hours the record had all the general features of *a*, except that the contractions were at a somewhat slower and more irregular rate and were higher. Luciani's groups with well-marked "stair-case" occurred from the ninth to the twenty-second hour. Only sixteen contractions occurred from the twentieth to the twenty-second hour of suspension in air. Ringer's solution with the calcium chloride increased to 0.04 per cent was used to wet the strip at the twenty-second hour, and was immediately followed by tall, regular

contractions for a period of over two hours. These contractions ceased quite abruptly. After a long pause — at the twenty-fifth hour — the strip was wet with drops of Ringer's solution containing 0.05 per cent calcium chloride. Immediately Luciani's groups appeared, with long intervening pauses. The groups became more and more frequent, and after three hours they passed into an irregular rhythm which continued twenty-five hours, thirty minutes. During the night the rate and height were both decreased by the low temperature, as in *a*. The calcium chloride was again increased in the Ringer's solution to 0.06 per cent, and at fifty-one hours, thirty minutes, the strip was given a two minutes' bath. The only effect was a slight increase in rate. Following the fiftieth hour of suspension the height of the contractions slowly decreased for twelve hours from 0.98 cm. to zero. Bathing the strip with Ringer's solutions containing 0.06 and 0.08 per cent calcium chloride, respectively, brought out contractions of good rate but only a millimetre in height (not recorded in the table below). At seventy-two hours strong electrical stimulation produced no observable contractions. At seventy-two hours, forty minutes, the strip was immersed in a bath of horse's serum, which produced contractions only 0.02 cm. to 0.03 cm. in height, *i. e.*, no recovery. The serum in this case, therefore, failed to cause an improvement in contractions. The experiment ended at the seventy-fourth hour of suspension in air.

Strip c. This strip in air contracted rapidly for a time but gradually became slower during twenty-four hours, forty-five minutes. Its contractions decreased in rate more gradually than in *a* or *b*, and Luciani's groups were not so prominent. Wetting the strip with Ringer's solution (calcium chloride 0.04 per cent) at twenty-four hours, forty-five minutes, was followed by an increase in the rate. After thirty hours, fifteen minutes, increase of calcium chloride to 0.05 per cent produced a slight increase in the rate. The height of the contractions slowly decreased from 0.69 cm. at the thirty-first hour to zero at the forty-second hour (see table below). The number of contractions for the last ten hours was very small. Further increase of calcium produced in each case comparatively rapid contractions, but no recovery of the height; thus, at the forty-second hour, the height of the contractions immediately following calcium chloride solution 0.06 per cent was 0.14 cm.; at the forty-fourth hour, following 0.08 per cent calcium chloride solution, it was 0.18 cm.; and at the forty-sixth hour, minute contractions were observed after 0.1 per cent calcium chloride. At the fiftieth hour the strip was given a bath of terrapin serum which produced contractions only 0.15 cm. high. No strong full contractions resulted.

I have made the amount of work given by these three strips of ventricular muscle while contracting automatically in a purely inorganic diet the basis of a series of calculations, in order to express the results in a way that admits of comparative study.

TABLE IV.

Tabulated results of Experiment 42 *a*, *b*, and *c*.

Time of suspension in air.	Number of contractions per hour.			Mean height of contractions during the hour = actual shortening of the muscle in centimetres.			Work done during one hour expressed as gramcentimetres.		
hour	a.	b.	c.	a.	b.	c.	a.	b.	c.
0–1	512	305	440	0.90	1.30	1.00	461	396	440
1–2	564	265	420	0.90	1.40	1.02	508	371	430
2–3	440	200	280	0.96	1.47	1.17	422	294	330
3–4	394	166	343	1.00	1.50	1.20	394	249	292
4–5	261	115	244	1.12	1.50	1.20	292	173	293
5–6	220	82	235	1.12	1.50	1.30	246	123	305
6–7	150	116	233	1.10	1.55	1.30	165	180	303
7–8	145	127	190	1.10	1.57	1.30	160	200	247
8–9 (12 P. M.)	98	44	114	1.15	1.57	1.37	113	69	157
9–10	100	64	110	1.15	1.60	1.40	115	102	154
10–10½	17	21	58	1.16	1.60	1.38	20	34	80
10½–17¼ [1]									
17¼–18	10	93	45	1.20	1.62	1.40	12	151	71
18–19	0	87	85	0	1.62	1.40	0	141	119
19–20	0	95	82	0	1.62	1.40	0	154	115
20–21 (12 M.)	18	9	82	1.30	1.50	1.42	23	13	117
21–22	22	7	84	1.30	1.50	1.40	25	11	118
22–23	7	165	60	1.32	1.57	1.32	9	260	80
23–24	0	190	33	0	1.59	1.22	0	300	40
24–25	3	44	21	1.30	1.59	1.20	4	70	25
25–26	40	40	20	1.38	1.57	1.10	55	63	22
26–27	45	16	32	1.38	1.58	1.05	62	25	34
27–28	110	34	35	1.38	1.57	0.95	152	54	33
28–29	115	98	78	1.35	1.58	0.87	155	154	68
29–30	138	50	129	1.33	1.57	0.70	183	79	90
30–31	161	60	45	1.28	1.58	0.69	205	95	31
31–32	120	67	56	1.28	1.50	0.65	153	100	36
32–33 (12 P. M.)	80	64	18	1.22	1.45	0.60	98	93	11
33–34	59	38	20	1.20	1.38	0.50	71	52	10

[1] Record lost for six hours, forty-five minutes.

TABLE IV. — *Continued.*

Time of suspension in air.	Number of contractions per hour.			Mean height of contractions during the hour = actual shortening of the muscle in centimetres.			Work done during one hour expressed as gram-centimetres.		
hour	a.	b.	c.	a.	b.	c.	a.	b.	c.
34–35	38	33	16	1.15	1.30	0.45	44	43	7
35–36	24	29	16	1.10	1.25	0.40	26	36	6
36–37	23	19	7	1.10	1.20	0.04	25	23	3
37–38	23	58	3	1.05	1.20	0.03	24	70	1
38–39	28	32	6	1.10	1.22	0.02	31	39	1
39–40	27	33	4	1.10	1.22	0.02	30	40	1
40–41	32	46	7	1.11	1.22	0.01	36	56	1
41–42	60	48	4	1.05	1.20	0.01	63	58	0
42–43	57	60	14	1.00	1.05	0.01	57	63	2
43–44	69	66	13	1.00	1.08	0.01	69	64	1
44–45 (12 M.)	88	34	19	0.98	1.05	0.02	86	36	4
45–46	80	37	28	0.90	1.05	0.01	72	39	3
46–47	83	37	23	0.80	1.05	0	66	39	0
47–48	112	37	50	0.70	1.02	0	78	38	0
48–49	154	42	75	0.55	1.00	0	85	42	0
49–50	78	42	trace	0.25	0.98	0	20	41	0
50–51	10	42		0.05	0.82		0	35	
51–52		16			0.75			12	
52–53		37			0.70			12	
53–54		40			0.60			26	
54–55		44			0.60			24	
55–56		55			0.62			34	
56–57 (12 P. M.)		48			0.60			29	
57–58		42			0.52			22	
58–59		45			0.45			20	
59–60		34			0.22			8	
60–61		34			0.07			2	
61–62		16			0.05			1	
Total	4817	3762	3877				4915	4958	4061

The strips of muscle were weighed as carefully as possible before suspending them, and also at the close of the treatment with inorganic salts. At each weighing the moisture was removed as nearly

as possible to the same extent by draining on glazed porcelain or on glass. The strips were then weighed between watch crystals. The results of all experiments where double weighings were made show a loss of weight. This fact is of significance, although I have not made a sufficient number of experiments for quantitative estimates from this standpoint.

TABLE V.

Weight of muscle strips at the beginning and end of treatment with Ringer's solution.

Number of the experiment.	Weight before treatment with Ringer's solution.	Weight after treatment with Ringer's solution.	Time of suspension in Ringer's solution.	Loss.
42 *a*	0.321 grams	0.196 grams	51½ hours	0.125 grams
42 *b*	0.283 "	0.231 "	72 "	0.052 "
42 *c*	0.340 "	0.232 "	50 "	0.108 "

The figures in this table, which express the weights before the use of Ringer's solution, are of the greatest importance for the present consideration. The three strips used gave respectively 4915, 4958, and 4061 gramcentimetres of recorded work. If this work be converted into its heat equivalent it may then be compared with the heat equivalents of the possible sources of energy-giving material. One gramcentimetre of work equals 980 ergs; one calorie equals 4.2×10^7 ergs; one gramcentimetre therefore equals $0.00002\frac{1}{3}$ calories.

The total heat of oxidation of one gram of proteid and of one gram of cane-sugar varies according to the determinations of different investigators. Stohman gives for beef (fat free) 5641, for urea 2465, and for cane-sugar 3959 calories respectively. Danilewsky gives for fibrin 5772, urea 2537, and for cane-sugar 4176 calories. Rubner gives for muscle extracted with water 5778, and for urea 2523 calories. If the oxidation equivalent of the urea formed as a result of the metabolism of one gram of proteid, *i.e.*, one third of a gram of urea, is deducted, it may be assumed that, in round numbers, the average oxidation energy of one gram of proteid or one gram of sugar available for muscular metabolism is 4890 calories for proteid, or 4000 calories for carbohydrate.

The amount of work recorded by the three strips is the equivalent of the oxidation of 0.0000233, 0.0000235, and 0.0000193 grams of

proteid or of 0.0000287, 0.0000289, 0.0000234 grams of carbohydrate respectively. These amounts represent the oxidation equivalents if all the energy appears as work, whereas it is well known that only a small fraction of the total energy of metabolism can be utilized as work. Gaule,[7] 1878, computed that, at least, not less than eight per cent of the energy of contraction in the frog's heart may be recorded as work. The well-known experiments of Fick upon striated muscles show that under the most favorable conditions twenty per cent may be regarded as a maximum yield in work. In the cardiac strip, it must be remembered that many of the fibres are cut across so that their contractions are lost, while others may by their contractions oppose those which exert a direct pull on the lever. It is, therefore, a liberal estimate to assume that fifteen per cent of the energy of oxidation may take the form of work during contractions of the ventricular strip, and that not more than two-thirds of this,—ten per cent of the total energy, is recovered on the record. These facts are arranged for comparison in the following table:—

TABLE VI.

Number of the experiment.	Work recorded.	Mechanical equivalent in heat.	Total oxidation equivalent in proteid.	Equivalent in proteid of metabolism if 10% is recovered as work.
42 *a*	4915 gr. cm.	0.11468 calories	0.0000233 grams	0.000233 grams
42 *b*	4958 "	0.11569 "	0.0000235 "	0.000235 "
42 *c*	4061 "	0.09312 "	0.0000193 "	0.000193 "

The average amount of serum-albumin in the serum of this species of terrapin during the winter season, as determined by Howell[21] in 1884, is 0.69 per cent. If the energy of the heart muscle is supplied by the metabolism of serum-albumin, as Kronecker holds, the amount of blood required to supply the necessary albumin for each of the three strips mentioned above must have been, respectively, 11.5, 13.1, and 9 per cent of their initial weights. But the percentage of blood in the entire body (determined upon mammals) is only 7.7 per cent, a large proportion of which is contained in the great vessels. The percentages exhibited in the following table reduce the question to an absurdity in so far as considering serum-albumin

as the source of the motor energy of the washed strips of the terrapin's heart is concerned.

TABLE VII.

Number of the experiment.	Work recorded.	Equivalent in the metabolism of serum-albumin of	Weight of serum necessary to supply the serum-albumin.	Initial weight of the muscles.	Proportion of serum in the muscles necessary to account for the work, etc.
42 *a*	4915 gr. cms.	0.000233 grams	0.0338 grams	0.321 grams	10.5%
42 *b*	4958 "	0.000235 "	0.0340 "	0.283 "	12.0%
42 *c*	4061 "	0.000193 "	0.0280 "	0.340 "	8.2%

The amount of paraglobulin in the blood of this terrapin is, according to Howell, comparatively large, 4.66 grams per 100 c.c. The equivalent of the above work may be expressed in terms of paraglobulin (Table VIII.).

TABLE VIII.

Number of the experiment.	Work recorded.	Equivalent in the metabolism of paraglobulin.	Weight of serum necessary to supply the paraglobulin.	Initial weight of the muscles.	Proportion of serum in the muscle strips necessary to supply the paraglobulin.
42 *a*	4915 gr. cms.	0.000233 grams	0.0050 grams	0.321 grams	1.6%
42 *b*	4958 "	0.000235 "	0.0050 "	0.283 "	1.8%
42 *c*	4061 "	0.000193 "	0.0041 "	0.340 "	1.2%

When it is remembered that the heart was irrigated with 0.7 per cent sodium chloride solution for one hour, and that the washing was facilitated by the contractions of the ventricle and by massage during this time, then the possibility of there being even this percentage of blood left in the capillaries or in the meshes of the tissues seems improbable.

The percentage of sugar in systemic blood varies from 0.1 per cent to 0.15 per cent. On this basis it would be utterly impossible to

account for the work, assuming that the energy came from the consumption of sugar furnished by the serum still left in the strips.

The more rational view applicable to this case is that the heart has stored material in its cells, and that the contractions of the isolated ventricular strips are at the expense of this material. The voluntary muscles are admittedly able to contract at the expense of stored contractile material. Why, therefore, should this function be denied to cardiac muscle?

The above experiment seems to demonstrate, beyond doubt, that *the terrapin's heart contracts at the expense of an antecedent contractile substance stored up in its own tissue.*

The amount of work developed by the heart strips under the influence of sodium chloride solution, or, in fact, any of the inorganic solutions used in these experiments, is perfectly represented by the comparison of the height and rate of contractions as discussed throughout this paper under the various headings, since the load was kept uniformly at one gram with all the levers used in the experiments quoted.

Exhaustion of Heart Muscle.

The word "exhaustion" as applied to the heart has been used to express many different states. At the present time it can no longer be employed without specifying the condition to which it applies. Before defining its use in this section, it will be interesting to sketch, briefly, its use in the literature on the subject.

In 1874 Kronecker and Stirling[3] found that a frog's heart filled with 0.6 per cent sodium chloride solution soon ceased to beat, and could be made to beat again only on the introduction of pure or diluted serum. They considered this condition brought on by sodium chloride solution one of exhaustion. From their experiments they were led to the conclusion that the heart in its contractions used material obtained directly from the blood. According to the view proposed by Kronecker and Stirling at that time, the frog's heart is exhausted in sodium chloride solution because the material of the blood necessary to each contraction is washed out.

Investigations tending to support and develop Kronecker's views were made by Stiénon,[6] 1878, Martius,[9] 1882, Kronecker and Popoff,[12] 1887, and White,[17] 1896.

In 1878 Gaule[7] showed that a heart which is quiet in sodium chloride could be made to contract for several hours by using a solu-

tion of alkali-saline, and that in this latter solution it gave off as many as one thousand contractions. This remarkable result can be explained, he says, only on the supposition that the muscle of the heart has the antecedent material which it uses in contractions stored up in its substance. When a heart will no longer beat in renewed alkaline sodium chloride solution, according to Gaule, it can be revived only by feeding it with blood. Apparently, according to his view, a heart is exhausted when its store of contractile material is used up.

In 1882 Martius,[9] working under the direction of Kronecker, ascribed the beneficial effect of alkali-saline not to a more complete utilization of stored material in the heart cells, as Gaule supposed, but to the fact that the alkali, by combining with carbon dioxide, prevented asphyxiation of the contractile tissue, and thus permitted a more complete utilization of the remnants of blood still in the interstices of the heart. Only when this material is used up is a heart truly exhausted. Martius stated that such a heart could be revived only by the use of substances like blood, serum, lymph, *i.e.*, substances containing serum-albumin, and he concluded that serum-albumin was the particular element in the blood used by the cardiac muscle in contraction.

In 1883 and 1885 Ringer[10] gave a new interpretation to the term "exhaustion," when he said "calcium salts are necessary for the proper contractions of the heart, yet they must be antagonized by potassium salts." According to this idea, a heart fails to contract in sodium chloride or in alkali-saline, because it is not supplied with the necessary calcium and potassium salts. Ringer's standpoint was still further emphasized by Howell and Cooke[15] in 1893, who showed that hearts that had ceased to beat after abundant irrigation with saline or alkali-saline could be revived and kept in normal contractions for long periods when supplied with Ringer's solution or with extracts of milk, blood, or gastric juice that contained only traces of proteid.

When in 1896 White[17] wrote in support of Kronecker's view he was compelled to take refuge in an hypothesis which attempts to overthrow the most painstaking work by one word. This hypothesis assumes that the heart may beat on infinitesimal, and, one may add, indetectible quantities of serum-albumin, and that it is almost if not quite impossible to completely remove all traces of serum-albumin from the muscular spaces of the heart.

White said that by the methods used by Merunowicz, Aubert, Martius, and other investigators, "it was impossible to completely wash out a heart." He held, therefore, that their conclusions were unfounded. He said that a heart must be irrigated through the most improved perfusion cannula, that it must be irrigated by successive solutions of sodium chloride, alkali-sodium chloride, and Ringer's solution until it is quiet. Only when it no longer responds to any of these solutions is it free from serum-albumin and truly exhausted. This is, however, the familiar argument in a circle, since the conclusion proceeds directly from the hypothesis assumed in the beginning. White also committed the grave error of not using the most favorable combination of salts in Ringer's solution, and, therefore, did not secure completely washed-out hearts in the sense in which he uses the term. By his own process of reasoning his results must be placed in the same category as those of the earlier investigators. In four examples that he gives, the hearts were exhausted after a total time of irrigation of: 1 hour, 25 minutes, 5 hours, 30 minutes, 9 hours, 15 minutes, and 4 hours, 45 minutes.

In this laboratory, in recent experiments upon the whole heart of the frog, the results of which will be published later, it has been observed that after the heart has ceased to beat upon the Ringer's mixture used by White it may still beat, and beat well, upon the mixture generally employed in this paper (0.7% NaCl, 0.026% $CaCl_2$, and 0.03% KCl). Moreover, with many hearts, although not with all, it has happened that after they have ceased to beat upon this last mixture the gradual increase of the amount of calcium salts in the Ringer's solution called forth new beats for a considerable period, an effect also obtained on heart strips, as previously described in this paper.

From the above review it will be seen that the term "exhaustion," as applied to the heart, either expresses states in which the inorganic salts necessary to the contractions of the heart are removed or disturbed in their relations, or it expresses states in which the antecedent organic contractile material is consumed or removed. In the first group may be included as many conditions of exhaustion as there are combinations of the inorganic salts that will not support contractions. That the series of beats obtained with any solution should disappear by successively smaller contractions, as they do in a heart filled with sodium chloride solution, is non-essential. In the second group must be included, on the one

hand, Kronecker's view, that exhaustion signifies a lack of sufficient free serum-albumin surrounding the muscle tissue to support contractions, and, on the other, Gaule's view, that exhaustion means a lack of antecedent contractile material stored in the cardiac tissue.

It seems to me it would be better to restrict the application of the term "exhaustion" to states of the cardiac tissue itself, and to designate in some other way all those conditions which imply the presence or absence of some substance or substances in the surrounding blood or artificial fluid. My own experiments are full of examples that demonstrate the inefficiency of sodium chloride solution to produce a true exhaustion in the ventricular strip. Time after time the sodium chloride solution series has been almost exactly duplicated on the same strip after an intervening recovery, due to a bath of solutions of inorganic salts alone. It is admitted that an inorganic diet cannot serve directly as a source of energy. My experiments give no convincing proof of the ability of the isolated muscle to use even the organic material of serum. In Experiment 42, given above, strips *a*, *b*, and *c* were made to contract incompletely with serum after they could no longer be aroused by Ringer's solution; but *b* gave only the slightest movements when treated with serum, and in both *a* and *c* the revival of contractions due to serum treatment did not last as long as did the contractions of *b*, still moist with the inorganic salt solution. It is possible, therefore, that the serum effect on *a* and *c* was due entirely to the more favorable relation of the inorganic salts, and produced only a more perfect exhaustion of the contractile material stored in the muscle cells of the strips.

The particular type of dying out of the contractions given by the strips in Experiment 42, quoted above, in which, after many hours, the amplitude of contractions slowly but steadily decreases to a zero from which no good recovery can be obtained, has occurred so often that it may be expected with confidence whenever muscular strips of the terrapin's ventricle are treated with any solution favorable to the development of continuous rhythmic contractions. In fact, in the very first experiment of this investigation, a heart strip, after seventy-two hours' continuous contraction, ceased in the same way as strips *a* and *b* of the above experiment. The strip of this first experiment was suspended in a muscle moist-chamber, and was made to begin its series of contractions by moistening it with serum much diluted with 0.6 per cent sodium chloride.

This type of dying out of the contractions I take to indicate a using up of the organic contractile material in the muscle. The completeness of exhaustion in this sense depends upon whether the muscular strip is subjected to the most favorable relation of the inorganic salts of the blood, especially of the sodium, potassium, and calcium salts. Whether or not the isolated strip is capable of being nourished, that is, of utilizing the stored energy of the organic constituents of the liquid in which it is immersed, is an independent question.

Summary.

1. A bath of normal serum will not keep a strip from the apex of terrapin ventricle in contraction, although it keeps it in good condition for contraction for three or four days. By slightly increasing the amount of calcium chloride in the serum regular contractions may be produced.

2. An artificial mixture of sodium, potassium, and calcium salts in the proportions in which they exist in serum acts like serum, in that it does not produce a continuous series of rhythmic contractions, but sustains the cardiac strip in good condition for contraction, for at least three days. For the terrapin's heart this proportion is approximately 0.7 per cent sodium chloride, 0.026 per cent calcium chloride, and 0.03 per cent potassium chloride.

3. Sodium chloride will produce and sustain contractions for a short time only, and the series of contractions presents the appearance of fatigue. This appearance of fatigue indicates only the removal of the inorganic salts necessary to contraction, and is not an exhaustion of the contractile substance of the muscle.

4. Calcium salts in isotonic solutions of sodium chloride stimulate the cardiac strip to increased rhythm and final permanent contracture.

5. Potassium chloride in isotonic solutions of sodium chloride prevents contractions, and keeps the ventricular strip in a state of relaxation.

6. There is an optimum ratio of the potash, calcium, and sodium salts in isotonic solution most favorable to the development and maintenance of the contractions in the ventricular strip. For the apex of the ventricle of the terrapin this proportion is sodium chloride 0.7 per cent, calcium chloride 0.04 or 0.05 per cent, potassium chloride 0.03 per cent, if the strip is fresh and filled with blood.

If the strip is from a heart that has been washed with 0.7 per cent sodium chloride, the proportion of salts given in conclusion 2, above, is the most favorable to the maintenance of contractions. The rhythm in the spongy ventricular strip is rarely perfectly regular in this solution.

7. Complete exhaustion of the contractile substance in the heart of the winter terrapin is brought about by the use of inorganic salt solution only after thirty to seventy-two hours' continuous rhythmic activity, or by seventy-two to one hundred hours' suspension, if the activity has been slight.

8. Cane sugar and urea in isotonic solutions do not produce rhythmic contractions in the isolated strip. Dextrose in isotonic solution throws the strip into strong tone and may produce an imperfect series of contractions.

REFERENCES.

[1] BROWN-SÉQUARD: Experimental researches applied to physiology and pathology, New York, 1853, p. 114.

[2] NASSE: Archiv f. d. ges. Physiol., 1869, ii, p. 118.

[3] KRONECKER and STIRLING: Beiträge zur Anatomie und Physiologie, Carl Ludwig gewidmet, 1874, p. 173.

[4] MERUNOWICZ: Arbeiten aus der physiol. Anstalt zu Leipzig, 1875, p. 132.

[5] MCGUIRE: Archiv f. Anat. u. Physiol., physiol. Abth., 1878, p. 321.

[6] STIÉNON: Archiv f. Anat. u. Physiol., physiol. Abth., 1878, p. 263.

[7] GAULE: Archiv f. Anat. u. Physiol., physiol. Abth., 1878, p. 291.

[8] AUBERT: Archiv f. d. ges. Physiol., 1881, xxiv, p. 357.

[9] MARTIUS: Archiv f. Anat. u. Physiol., physiol. Abth., 1882, p. 543.

[10] RINGER: Journal of physiology, 1883, iv, p. 29. See also *ibid.*, 1883, iv, p. 370; 1884, v, p. 352; 1885, vi, p. 154; 1886, vii, p. 291; 1887, viii, p. 20 and p. 288; 1893, xiv, p. 125; 1894, xvi, p. 1; and 1895, xviii, p. 425.

[11] VON OTT: Archiv f. Anat. u. Physiol., physiol. Abth., 1883, p. 1.

[12] KRONECKER and POPOFF: Archiv f. Anat. u. Physiol., physiol. Abth., 1887, p. 345. Zeitschrift für Biologie, 1888, xxv, p. 427.

[13] BRINCK and KRONECKER: Archiv f. Anat. u. Physiol., physiol. Abth., 1887, p. 347.

[14] HEFFTER: Archiv f. exper. Pathol. u. Pharmakol., 1892, xxix, s. 41.

[15] HOWELL and COOKE: Journal of physiology, 1893, xiv, p. 198.

[16] LOCKE: Journal of physiology, 1895, xviii, p. 319.

[17] WHITE: Journal of physiology, 1896, xix, p. 344.

[18] BERKLEY: Johns Hopkins hospital reports, 1895, iv, p. 248.

[19] JONES and MACKAY: Zeitschr. f. physikalische Chemie, 1897, xvii, p. 2.

[20] GERLACH: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1873, p. 99.

[21] HOWELL: Studies from the biological laboratory of the Johns Hopkins University, 1884, p. 49.